

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C.371**U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
09/646561INTERNATIONAL APPLICATION
PCT/US99/06187INTERNATIONAL FILING DATE
19 March 1999 (19.03.99)PRIORITY DATE CLAIMED
19 March 1998 (19.03.98)

TITLE OF INVENTION

T CELL COSTIMULATORY PROTEINS, SEQUENCES AND USES THEREOF

APPLICANT(S) FOR DO/EO/US

Gek-Kee SIM; Shumin YANG; and Karen S. SELLINS

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C.371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371 (f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371 (b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) **(unexecuted) (4 sheets);**
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included
13. ☐ A FIRST preliminary amendment.
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: **Transmittal Letter (2 sheets in duplicate); Transmittal Letter for Sequence Listing along with Sequence listing Diskette attached (1 sheet); PCT International Published Application (WO 99/47558) (without International Search Report) (147 sheets); Cover Sheet of PCT International Published Application (WO 99/47558) (with International Search Report attached) (7 sheets); Certificate of Express Mailing (1 sheet); and Return Postcard.**

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/646561		INTERNATIONAL APPLICATION NO. PCT/US99/06187		ATTORNEY'S DOCKET NO. HKZ-029CPUS	
---	--	--	--	---	--

17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) .(a/o January 1, 2000): Search Report has been prepared by the EPO or JPO.....\$970 International preliminary examination fee paid to USPTO (37 CFR 1.482).....\$840 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....\$690 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....\$670 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....\$96 <div style="text-align: right; margin-right: 50px;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>				CALCULATIONS PTO USE ONLY	
				\$970	
Surcharge of \$130.00 for furnishing the oath or declaration later than 20 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$--	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	109 -20 =	89	X \$18.00	\$1602	
Independent claims	9 -3 =	6	X \$78.00	\$468	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ 260.00	\$260
TOTAL OF ABOVE CALCULATIONS =				\$3300	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28)				\$--	
SUBTOTAL =				\$3300	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$--	
TOTAL NATIONAL FEE =				\$3300	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$--	
TOTAL FEES ENCLOSED =				\$--	
				Amount to be: refunded	\$
				charged	\$

a. ☐ A check in the amount of \$_____ to cover the above fees is enclosed.

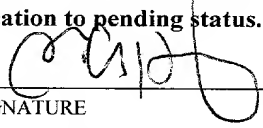
b. ☒ Please charge my Deposit Account No. **12-0080** in the amount of **\$3300** to cover the above fees.
A duplicate copy of this sheet is enclosed.

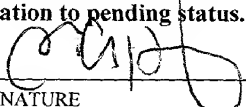
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit
any overpayment to Deposit Account No. **12-0080**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Elizabeth A. Hanley, Esq.
LAHIVE & COCKFIELD, LLP
28 State Street
Boston, Massachusetts 02109
United States of America
(617)227-7400
Date: 19 September 2000


 SIGNATURE
Elizabeth A. Hanley
 NAME
33,505
 REGISTRATION NUMBER

APPLICATION NO. (if known, see 37 CFR 1.5) 097617		INTERNATIONAL APPLICATION NO PCT/US99/06187		ATTORNEY'S DOCKET NO HKZ-029CPUS	
U.S. APPLICANT'S FEE (37 CFR 1.492 (a) (1) - (5)) (a/o January 1, 2000):				CALCULATIONS PTO USE ONLY	
International preliminary examination fee paid to USPTO (37 CFR 1.482).....					
International preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....					
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....					
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....					
ENTER APPROPRIATE BASIC FEE AMOUNT =					
Surcharge of \$130.00 for furnishing the oath or declaration later than 20 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$--	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	109 -20 =	89	X \$18.00	\$1602	
Independent claims	9 -3 =	6	X \$78.00	\$468	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ 260.00	\$260	
TOTAL OF ABOVE CALCULATIONS =				\$3300	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28)				\$--	
SUBTOTAL =				\$3300	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$--	
TOTAL NATIONAL FEE =				\$3300	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$--	
TOTAL FEES ENCLOSED =				\$--	
				Amount to be:	\$
				refunded	
				charged	\$
a. <input type="checkbox"/> A check in the amount of \$ _____ to cover the above fees is enclosed.					
b. <input checked="" type="checkbox"/> Please charge my Deposit Account No. <u>12-0080</u> in the amount of <u>\$3300</u> to cover the above fees. A duplicate copy of this sheet is enclosed.					
c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>12-0080</u> . A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: Elizabeth A. Hanley, Esq. LAHIVE & COCKFIELD, LLP 28 State Street Boston, Massachusetts 02109 United States of America (617)227-7400 Date: 19 September 2000					
 SIGNATURE Elizabeth A. Hanley NAME 33,505 REGISTRATION NUMBER					

NOVEL FORMS OF T CELL COSTIMULATORY PROTEINS, NUCLEIC ACID
MOLECULES, AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to B7 and CTLA4 nucleic acid molecules, proteins
5 encoded by such nucleic acid molecules, antibodies raised against such proteins and
inhibitors of such proteins or nucleic acid molecules. The present invention also
includes therapeutic compositions comprising such nucleic acid molecules, proteins,
antibodies and/or inhibitors, as well as their use to regulate an immune response in an
animal.

10 BACKGROUND OF THE INVENTION

T lymphocytes (i.e., T cells) play a key role in regulating immune responses in
animals. Activation of T cells requires two signals delivered by antigen presenting cells
to non-activated T cells. The first, or primary, signal is mediated by the interaction of
the T cell receptor/CD3 complex (TcR/CD3) with MHC-associated antigenic peptide.
15 The second, or costimulatory, signal regulates the T cell proliferative response and
induction of effector functions. Costimulatory signals determine whether a T cell will be
activated or inactivated to a state of tolerance. Molecules present on the surface of
antigen presenting cells which are involved in T cell costimulation include B7
molecules. As known in the art, B7 molecules include two forms, B7-1 and B7-2, also
20 known as CD80 and CD86, respectively. These molecules are counter-receptors for two
ligands, CD28 and CTLA4. Both B7-1 and B7-2 bind both CD28 and CTLA4. Upon
binding to CD28 or CTLA4, mouse B7 can cause T cells to proliferate and secrete
interleukin-2 in conjunction with engagement of T cell receptor with a major
histocompatibility molecule complexed with peptide.

25 A full-length B7 protein is composed of several regions including, from the N-
terminus, a signal peptide, an extracellular region, a transmembrane region, and a
cytoplasmic region. The predicted amino acid sequence of B7-1 shares homology with
members of the immunoglobulin (Ig) superfamily due to the presence of two contiguous
Ig-like domains in the extracellular region. For example, in the mature mouse B7-1
30 protein, residues 1-112 share homology with the Ig variable (IgV) domain and residues
113-210 shares homology with Ig constant (IgC) domain (Freeman et al., *J. Immunol.*,

Vol. 143, pp. 2714-2722, 1989). B7-2 was also found to have similar structural features in the extracellular region. Similarly, CD28 and CTLA4 each have one IgV-like domain in the extracellular region.

Prior investigators have disclosed sequences encoding: human B7-1 domains are
5 encoded by distinct exons in their respective genes as described for human B7-1
(Freeman et al., *J. Immunol.*, vol. 143, pp. 2714-2722, 1989); human B7 (Azuma et al.,
Nature, vol. 366, pp. 76-79, 1993; or Selvakumar et al., *Immunogenetics.*, vol. 36, pp.
175-181, 1992); rhesus monkey B7-1 (Villinger et al., *J. Immunol.*, vol. 155, pp. 3946-
3954, 1995); cat B7-1 (Hash et al., Thesis, Veterinary Pathobiology, Texas A & M,
10 1996); rabbit B7-1 (Isono et al., *Immunogenetics.*, vol. 42, pp. 217-220, 1995); rat B7-1
(Judge et al., *Intl. Immunol.*, vol. 7, pp. 171-178, 1995; Jackerott et al., Genbank
Accession No. U10925, 1994); mouse B7-1 (Borriello et al., *J. Immunol.*, vol. 153, pp.
5038-5048, 1994); human B7-2 (Freeman et al., *Science*, vol. 262, pp. 909-911, 1993);
mouse B7-2 (Freeman et al., *J. Expt. Med.*, vol. 178, pp. 2185-2192, 1993; or) and rat B7
15 genes (Judge et al., *Intl. Immunol.*, vol. 7, pp. 171-178, 1995; Goodman, Genbank
Accession No. U31330, 1995).

Prior investigators have also disclosed sequences encoding: mouse CTLA4
(Brunet et al., *Nature*, vol. 328, pp. 267-270, 1987); human CTLA4 (Dariavach et al.,
Eur J Immunol, vol 18, pp. 1901-1905, 1988); rabbit CTLA4 (Isono and Seto,
20 *Immunogenetics*, vol. 42, pp. 217-220, 1995); rat CTLA4 (Oaks et al., *Immunogenetics*,
vol. 43, pp. 173-174, 1996); and bovine CTLA4 (Parsons et al., *Immunogenetics*, vol. 43,
pp. 388-391, 1996).

Messenger RNA of different sizes have been identified for B7-2 genes by
Northern blot hybridization (Inobe et al., *Biochem. Biophys. Res. Communic.*, vol. 200,
25 pp. 443-449, 1994; or Boriello et al., *J. Immunol.*, vol. 155, pp. 5490-5497, 1995).
These B7-2 mRNA species have been assumed to be generated through alternative
splicing or differential use of polyadenylation sites.

There remains a need for compounds and methods to regulate an immune
response by manipulation of the function of B7-1 and/or B7-2.

SUMMARY OF THE INVENTION

The present invention relates to B7 and CTLA4 nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins and inhibitors of such proteins or nucleic acid molecules. The inventors have discovered
5 novel naturally-occurring variants of B7 proteins that are produced by, for example, alternative RNA splicing or alternative termination of full-length amino acid sequence. The inventors are the first to discover such variants and therefore disclose novel therapeutic compositions and methods to use such variants to regulate an immune response in an animal useful in the treatment of diseases including cancer, infectious
10 diseases, inflammation or allergy. Thus, the present invention also includes therapeutic compositions comprising such nucleic acid molecules, proteins, antibodies and/or inhibitors, as well as their use to regulate an immune response in an animal.

One embodiment of the present invention is an isolated nucleic acid molecule having a nucleic acid sequence that is at least about 80 percent identical to a nucleic acid
15 sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28 and SEQ ID NO:29, or a fragment thereof having at least 12 nucleotides.

20 Another embodiment of the present invention is an isolated nucleic acid molecule consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, and SEQ ID NO:40.

Another embodiment is a nucleic acid molecule having a nucleic acid sequence that is at least about 90 percent identical to a nucleic acid sequence selected from the
25 group consisting of SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, or a fragment thereof having at least about 12 nucleotides.

Another embodiment of the present invention is an isolated nucleic acid molecule selected from the group consisting of: (a) a nucleic acid molecule having a
30 nucleic acid sequence encoding a B7 protein selected from the group consisting of a protein having an amino acid sequence that is at least about 80 percent identical to the

amino acid sequence SEQ ID NO:2, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:2, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:7, a protein

5 comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:7, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:12, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid

10 sequence SEQ ID NO:12, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID

15 NO:26, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:26, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:34, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino

20 acid sequence SEQ ID NO:34, and a protein having amino acid sequence SEQ ID NO:37; (b) a nucleic acid molecule comprising a complement of any of said nucleic acid sequences set forth in (a); (c) a nucleic acid molecule having a nucleic acid sequence encoding a CTLA4 protein selected from the group consisting of: a protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence

25 selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; and a protein comprising an epitope of said protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; and (d) a nucleic acid molecule comprising a complement of any of said nucleic acid sequences set forth in (c); wherein said B7

30 protein elicits an immune response against a naturally-occurring B7 protein, and wherein

said CTLA4 protein elicits an immune response against a naturally-occurring CTLA4 protein.

The present invention also includes methods to produce B7 proteins recombinantly using such nucleic acid molecules.

5 Yet another embodiment of the present invention is an isolated nucleic acid molecule that encodes a naturally-occurring soluble mammalian B7 protein, and complements thereof. A preferred nucleic acid molecule includes a nucleic acid molecule comprising a nucleic acid sequence encoding a naturally-occurring soluble B7-2 protein having extracellular and intracellular domains but lacking at least a portion of
10 the transmembrane domain sufficient to produce a soluble protein upon translation of the nucleic acid molecule in a suitable host cell. Another preferred nucleic acid molecule includes a nucleic acid molecule comprising a nucleic acid sequence encoding a naturally-occurring soluble B7-1 protein having an extracellular domain but lacking at least a portion of the transmembrane and intracellular domains sufficient to produce a
15 soluble protein upon translation of the nucleic acid molecule in a suitable host cell. Yet another preferred nucleic acid molecule comprises a nucleic acid sequence encoding a naturally-occurring soluble feline B7-1 protein having an extracellular domain but lacking at least a portion of transmembrane and intracellular domains sufficient to produce a soluble protein upon translation of said nucleic acid molecule in a suitable
20 host cell, wherein said extracellular domain comprises an IgV-like domain, but lacks an IgC-like domain.

The present invention also includes (a) an isolated protein comprising a B7 protein selected from the group consisting of a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:2, a
25 protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:2, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:7, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid
30 sequence SEQ ID NO:7, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:12, a protein comprising an

epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:12, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:26, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:26, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:34, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:34, and a protein consisting of amino acid sequence SEQ ID NO:37, wherein said B7 protein elicits an immune response against a naturally-occurring B7 protein; and (b) an isolated protein comprising a CTLA4 protein selected from the group consisting of: a protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; and a protein comprising an epitope of said protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; wherein said CTLA4 protein elicits an immune response against a naturally-occurring CTLA4 protein..

The present invention also includes an isolated naturally-occurring soluble mammalian B7 protein. A preferred naturally-occurring soluble mammalian B7 protein comprises an isolated naturally-occurring soluble B7-2 protein comprising extracellular and intracellular domains but lacking at least a portion of the transmembrane domain of the B7-2 protein sufficient to be a soluble B7-2 protein. Another preferred naturally-occurring soluble mammalian B7 protein comprises an isolated naturally-occurring soluble B7-1 protein comprising the extracellular domain but lacking at least a portion of the transmembrane and intracellular domains of the B7-1 protein sufficient to produce a soluble B7-1 protein. Another preferred naturally-occurring soluble mammalian B7 protein comprises an isolated naturally-occurring soluble feline B7-1 protein having an

extracellular domain but lacking at least a portion of transmembrane and intracellular domains sufficient to produce a soluble protein upon translation of said nucleic acid molecule in a suitable host cell, wherein said extracellular domain comprises an IgV-like domain, but lacks an IgC-like domain.

- 5 One aspect of the present invention is a therapeutic composition that, when administered to an animal, regulates T cell mediated immune responses in said animal, said therapeutic composition comprising a therapeutic compound selected from the group consisting of: an isolated protein comprising a B7 protein, wherein said B7 protein is selected from the group consisting of a protein having an amino acid sequence that is
- 10 at least about 80 percent identical to the amino acid sequence SEQ ID NO:2, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:2, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid
- 15 sequence SEQ ID NO:7, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:7, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:12, a protein comprising an epitope of
- 20 said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:12, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein comprising an epitope of said protein having an amino acid sequence that is at least
- 25 about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:26, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid
- 30 sequence SEQ ID NO:26, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:34, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:34, a protein having amino acid
- sequence SEQ ID NO:37, an isolated naturally-occurring soluble B7 protein; a mimotope of any of said B7 proteins; a multimeric form of any of said B7 proteins; an isolated

protein comprising a CTLA4 protein selected from the group consisting of: a protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; and a protein comprising an epitope of said protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; a mimotope of any of said CTLA4 proteins; a multimeric form of any of said CTLA4 proteins; an isolated nucleic acid molecule selected from the group consisting of: a nucleic acid molecule having a nucleic acid sequence that is at least about 80 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:35; a nucleic acid molecule consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, and SEQ ID NO:40; and a nucleic acid molecule having a nucleic acid sequence that is at least about 90 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, and SEQ ID NO:50; an isolated antibody that selectively binds to any of said B7 proteins; an inhibitor of B7 protein activity identified by its ability to inhibit the activity of any of said B7 proteins; an isolated antibody that selectively binds to any of said CTLA4 proteins; and an inhibitor of CTLA4 protein activity identified by its ability to inhibit the activity of any of said CTLA4 proteins, and a mixture thereof. The present invention also includes methods to administer such therapeutic compositions.

Yet another aspect of the present invention is a method to identify a compound capable of regulating T cell mediated immune responses in an animal, said method comprising: (a) contacting an isolated B7 or CTLA4 protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12, SEQ ID NO:17, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:34, SEQ ID

NO:42, and SEQ ID NO:47 with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein has T cell stimulating activity; and (b) determining if the putative inhibitory compound inhibits said activity.

DETAILED DESCRIPTION OF THE INVENTION

5 The present invention provides for isolated B7 and CTLA4 proteins, isolated B7 and CTLA4 nucleic acid molecules, antibodies directed against B7 and CTLA4 proteins, and compounds derived therefrom that regulate the immune response of an animal (e.g. inhibitors, antibodies and peptides). Identification of canine B7 nucleic acid molecules of the present invention is unexpected because initial attempts to obtain nucleic acid
10 molecules using PCR were unsuccessful. After numerous attempts, the inventors discovered specific primers that were useful for isolating such nucleic acid molecules. In addition, the inventors discovered novel naturally-occurring nucleic acid molecules that encode variable forms of B7-1 and B7-2 proteins.

 A B7 protein can refer to a B7-1 protein, a B7-2 protein, including variants
15 thereof. As used herein, the phrase "regulate an immune response" refers to modulating the activity of cells involved in an immune response. The term "regulate" can refer to increasing or decreasing an immune response. Regulation of an immune response can be determined using methods known in the art as well as methods disclosed herein. As used herein, the terms isolated B7 proteins, isolated CTLA4 proteins, isolated B7
20 nucleic acid molecules and isolated CTLA4 nucleic acid molecules refers to B7 and CTLA4 proteins and B7 and CTLA4 nucleic acid molecules derived from mammals and, as such, can be obtained from their natural source, or can be produced using, for example, recombinant nucleic acid technology or chemical synthesis. Also included in the present invention is the use of these proteins, nucleic acid molecules, antibodies, and
25 compounds derived therefrom as therapeutic compositions to regulate the immune response of an animal as well as in other applications, such as those disclosed below.

 One embodiment of the present invention is an isolated protein that includes a B7 protein or a CTLA4 protein. It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, a protein refers to one or more proteins or at least
30 one protein. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising",

"including", and "having" can be used interchangeably. According to the present invention, an isolated, or biologically pure, protein, is a protein that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the protein has been purified. An isolated protein of the present invention can be obtained from its natural source, can be produced using recombinant DNA technology, or can be produced by chemical synthesis.

As used herein, an isolated B7 or CTLA4 protein of the present invention (i.e., a B7 or CTLA4 protein) can be a full-length protein or any homolog of such a protein. An isolated protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to elicit an immune response against a B7 or CTLA4 protein, bind to CD28 or CTLA4 (for a B7 protein), or bind to B7 (for a CTLA4 protein), or stimulate T cell activity. Examples of B7 and CTLA4 homologs include B7 and CTLA4 proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog includes at least one epitope capable of eliciting an immune response against a B7 or CTLA4 protein, of binding to an antibody directed against a B7 or CTLA4 protein, of binding to CD28 or CTLA4 (for a B7 protein), of binding to B7 (for a CTLA4 protein) and/or of stimulating T cell activity. That is, when the homolog is administered to an animal as an immunogen, using techniques known to those skilled in the art, the animal will produce an immune response against at least one epitope of a natural B7 or CTLA4 protein. The ability of a protein to effect an immune response can be measured using techniques known to those skilled in the art. As used herein, the term "epitope" refers to the smallest portion of a protein capable of selectively binding to the antigen binding site of an antibody, or to B7, CD28 or CTLA4. It is well accepted by those skilled in the art that the minimal size of a protein epitope capable of selectively binding to the antigen binding site of an antibody is about six to seven amino acids.

B7 and CTLA4 protein homologs can be the result of natural allelic variation, including natural mutation. B7 and CTLA4 protein homologs of the present invention can also be produced using techniques known in the art including, but not limited to,

direct modifications to the protein or modifications to the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

B7 and CTLA4 proteins of the present invention include variants of a full-length B7 or CTLA4 protein. Such variants include B7 or CTLA4 proteins that are less than full-length. As used herein variants of the present invention refer to nucleic acid molecules that are naturally-occurring as defined below, and may result from alternative RNA splicing, alternative termination of an amino acid sequence or DNA recombination. Examples of variants include allelic variants as defined below, or soluble forms of a B7 or CTLA4 protein.

B7 and CTLA4 proteins of the present invention are encoded by B7 and CTLA4 nucleic acid molecules. As used herein, a B7 nucleic acid molecule includes nucleic acid sequences related to natural B7 gene, and preferably, to a B7-1 or B7-2 gene. As used herein, a CTLA4 nucleic acid molecule includes nucleic acid sequences related to natural CTLA4 gene. As used herein, a B7 or CTLA4 gene refers to the natural genomic elements that encode a B7 or CTLA4 protein, and includes all regions such as regulatory regions that control production of the B7 or CTLA4 protein encoded by the gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself, and any introns or non-translated coding regions. As used herein, a gene that "includes" or "comprises" a sequence may include that sequence in one contiguous array, or may include the sequence as fragmented exons. As used herein, the term "coding region" refers to a continuous linear array of nucleotides that translates into a protein. A full-length coding region is that region that is translated into a full-length, i.e., a complete, protein as would be initially translated in its natural milieu, prior to any post-translational modifications.

In one embodiment, a B7 gene of the present invention includes the nucleic acid sequence SEQ ID NO:1, as well as the complement of SEQ ID NO:1. Nucleic acid sequence SEQ ID NO:1 represents the deduced sequence of the coding strand of a cDNA (complementary DNA) denoted herein as nucleic acid molecule nCaB7-1₂₈₃₀, the production of which is disclosed in the Examples. Nucleic acid molecule nCaB7-1₂₈₃₀ comprises an apparently full-length coding region of canine B7-1. The complement of

SEQ ID NO:1 (represented herein by SEQ ID NO:3) refers to the nucleic acid sequence of the strand complementary to the strand having SEQ ID NO:1, which can easily be determined by those skilled in the art. Likewise, a nucleic acid sequence complement of any nucleic acid sequence of the present invention refers to the nucleic acid sequence of the nucleic acid strand that is fully complementary to (i.e., can form a double helix with) the strand for which the sequence is cited. It should be noted that since nucleic acid sequencing technology is not entirely error-free, SEQ ID NO:1 (as well as other nucleic acid and protein sequences presented herein) represents an apparent nucleic acid sequence of the nucleic acid molecule encoding a B7 protein of the present invention.

In another embodiment, a B7 gene of the present invention includes the nucleic acid sequence SEQ ID NO:6, as well as the complement represented by SEQ ID NO:8. Nucleic acid sequence SEQ ID NO:6 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nCaB7-2₁₈₉₇, the production of which is disclosed in the Examples. Nucleic acid molecule nCaB7-2₁₈₉₇ comprises an apparently full-length coding region of canine B7-2.

In another embodiment, a B7 gene of the present invention includes the nucleic acid sequence SEQ ID NO:11, as well as the complement represented by SEQ ID NO:13. Nucleic acid sequence SEQ ID NO:11 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nCaB7-1s₁₀₂₄, the production of which is disclosed in the Examples. Nucleic acid molecule nCaB7-1s₁₀₂₄ comprises an apparently full-length coding region of a naturally-occurring soluble canine B7-1. In another embodiment, a B7 gene of the present invention includes the nucleic acid sequence SEQ ID NO:16, as well as the complement represented by SEQ ID NO:18. Nucleic acid sequence SEQ ID NO:16 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nCaB7-2s₁₇₉₅, the production of which is disclosed in the Examples. Nucleic acid molecule nCaB7-2s₁₇₉₅ comprises an apparently full-length coding region of a naturally-occurring soluble canine B7-2.

In another embodiment, a B7 gene of the present invention includes the nucleic acid sequence SEQ ID NO:25, as well as the complement represented by SEQ ID NO:27. Nucleic acid sequence SEQ ID NO:25 represents the deduced sequence of the

coding strand of a cDNA denoted herein as nucleic acid molecule nFeB7-2₁₈₉₇, the production of which is disclosed in the Examples. Nucleic acid molecule nFeB7-2₁₈₉₇ comprises an apparently full-length coding region of feline B7-2.

In another embodiment, a B7 gene of the present invention includes the nucleic acid sequence SEQ ID NO:36, as well as the complement represented by SEQ ID NO:38. Nucleic acid sequence SEQ ID NO:36 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeB7-1s₅₉₄, the production of which is disclosed in the Examples. Nucleic acid molecule nFeB7-1s₅₉₄ comprises an apparently full-length coding region of a naturally-occurring soluble feline B7-1.

In another embodiment, a CTLA4 gene of the present invention includes the nucleic acid sequence SEQ ID NO:41, as well as the complement represented by SEQ ID NO:43. Nucleic acid sequence SEQ ID NO:41 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nCaCTLA4₁₈₅₆, the production of which is disclosed in the Examples. Nucleic acid molecule nCaCTLA4₁₈₅₆ comprises an apparently full-length coding region of a canine CTLA4. In another embodiment, a CTLA4 gene of the present invention includes the nucleic acid sequence SEQ ID NO:46, as well as the complement represented by SEQ ID NO:48. Nucleic acid sequence SEQ ID NO:46 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeCTLA4₁₈₈₃, the production of which is disclosed in the Examples. Nucleic acid molecule nFeCTLA4₁₈₈₃ comprises an apparently full-length coding region of a feline CTLA4.

In another embodiment, a B7 or CTLA4 gene or nucleic acid molecule can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50 or any other B7 nucleic acid

- 14 -

sequence cited herein. An allelic variant of a B7 or CTLA4 gene including the particular SEQ ID NO's cited herein, is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including the particular SEQ ID NO's cited herein, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Because natural selection typically selects against alterations that affect function, allelic variants usually encode proteins having similar activity to that of the protein encoded by the gene to which they are being compared. Allelic variants of genes or nucleic acid molecules can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions), or can involve alternative splicing of a nascent transcript, thereby bringing alternative exons into juxtaposition. Allelic variants are well known to those skilled in the art and would be expected to be found within a given animal, since the respective genomes are diploid, and sexual reproduction will result in the reassortment of alleles.

The minimal size of a B7 or CTLA4 protein homolog of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid (i.e., hybridize under stringent hybridization conditions) with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. As used herein, "stringent hybridization conditions" refer to those experimental conditions under which nucleic acid molecules having similar nucleic acid sequences will anneal to each other. Stringent hybridization conditions are well known to those of skill in the art. Such standard conditions are disclosed, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989. Stringent hybridization conditions typically permit the hybridization of nucleic acid molecules having at least about 70% nucleic acid sequence identity with the nucleic acid molecule being used as a probe in the hybridization reaction. Formulae to calculate the appropriate hybridization and wash conditions to achieve hybridization permitting 30% or less mis-match between two nucleic acid molecules are disclosed, for example, in Meinkoth et al, 1984, *Anal. Biochem* 138, 267-284. As such, the size of the nucleic acid molecule encoding such a protein homolog is dependent on nucleic acid composition and percent homology between the nucleic acid molecule and complementary sequence. It should also be noted that the extent of homology required to form a stable hybrid can

vary depending on whether the homologous sequences are interspersed throughout the nucleic acid molecules or are clustered (i.e., localized) in distinct regions on the nucleic acid molecules. The minimal size of such nucleic acid molecules is typically at least about 12 to about 15 nucleotides in length if the nucleic acid molecules are GC-rich and at least about 15 to about 17 bases in length if they are AT-rich. As such, the minimal size of a nucleic acid molecule used to encode a B7 protein homolog of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of a B7 protein homolog of the present invention is from about 4 to about 6 amino acids in length. There is no limit, other than a practical limit, on the maximal size of such a nucleic acid molecule in that the nucleic acid molecule can include a portion of a gene, an entire gene, or multiple genes, or portions thereof. The preferred size of a protein encoded by a nucleic acid molecule of the present invention depends on whether a full-length, fusion, multivalent, or functional portion of such a protein is desired.

Stringent hybridization conditions are determined based on defined physical properties of the gene to which the nucleic acid molecule is being hybridized, and can be defined mathematically. Stringent hybridization conditions are those experimental parameters that allow an individual skilled in the art to identify significant similarities between heterologous nucleic acid molecules. These conditions are well known to those skilled in the art. See, for example, Sambrook, *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, and Meinkoth, *et al.*, 1984, *Anal. Biochem.* 138, 267-284. As explained in detail in the cited references, the determination of hybridization conditions involves the manipulation of a set of variables including the ionic strength (M, in moles/liter), the hybridization temperature (°C), the concentration of nucleic acid helix destabilizing agents (such as formamide), the average length of the shortest hybrid duplex (n), and the percent G + C composition of the fragment to which an unknown nucleic acid molecule is being hybridized. For nucleic acid molecules of at least about 150 nucleotides, these variables are inserted into a standard mathematical formula to calculate the melting temperature, or T_m , of a given nucleic acid molecule. As defined in the formula below, T_m is the temperature at which two complementary nucleic acid molecule strands will disassociate, assuming 100% complementarity between the two strands:

$$T_m = 81.5^{\circ}\text{C} + 16.6 \log M + 0.41(\%G + C) - 500/n - 0.61(\%\text{formamide}).$$

For nucleic acid molecules smaller than about 50 nucleotides, hybrid stability is defined by the dissociation temperature (T_d), which is defined as the temperature at which 50% of the duplexes dissociate. For these smaller molecules, the stability at a standard ionic strength is defined by the following equation:

$$T_d = 4(G + C) + 2(A + T).$$

A temperature of 5°C below T_d is used to detect hybridization between perfectly matched molecules.

Also well known to those skilled in the art is how base pair mismatch, i.e. differences between two nucleic acid molecules being compared, including non-complementarity of bases at a given location, and gaps due to insertion or deletion of one or more bases at a given location on either of the nucleic acid molecules being compared, will affect T_m or T_d for nucleic acid molecules of different sizes. For example, T_m decreases about 1°C for each 1% of mismatched base pairs for hybrids greater than about 150 bp, and T_d decreases about 5°C for each mismatched base pair for hybrids below about 50 bp. Conditions for hybrids between about 50 and about 150 base pairs can be determined empirically and without undue experimentation using standard laboratory procedures well known to those skilled in the art. These simple procedures allow one skilled in the art to set the hybridization conditions (by altering, for example, the salt concentration, the formamide concentration or the temperature) so that only nucleic acid hybrids with greater than a specified % base pair mismatch will hybridize. Stringent hybridization conditions are commonly understood by those skilled in the art to be those experimental conditions that will allow about 30% base pair mismatch (i.e., about 70% identity). Because one skilled in the art can easily determine whether a given nucleic acid molecule to be tested is less than or greater than about 50 nucleotides, and can therefore choose the appropriate formula for determining hybridization conditions, he or she can determine whether the nucleic acid molecule will hybridize with a given gene under stringent hybridization conditions and similarly whether the nucleic acid molecule will hybridize under conditions designed to allow a desired amount of base pair mismatch.

Hybridization reactions are often carried out by attaching the nucleic acid molecule to be hybridized to a solid support such as a membrane, and then hybridizing with a labeled nucleic acid molecule, typically referred to as a probe, suspended in a hybridization solution. Examples of common hybridization reaction techniques include, but are not limited to, the well-known Southern and northern blotting procedures. Typically, the actual hybridization reaction is done under non-stringent conditions, i.e., at a lower temperature and/or a higher salt concentration, and then high stringency is achieved by washing the membrane in a solution with a higher temperature and/or lower salt concentration in order to achieve the desired stringency.

- For example, if the skilled artisan wished to identify a nucleic acid molecule that hybridizes under stringent hybridization conditions with a specific or known canine nucleic acid molecule of about 150 bp in length, the following conditions could preferably be used. The average G + C content of canine DNA includes about 35%, about 36%, about 37%, about 38%, about 39%, about 41%, about 42%, about 43%, about 44%, about 45%, with about 40% being preferred. The unknown nucleic acid molecules would be attached to a support membrane, and the specified 150 bp nucleic acid molecule would be labeled, e.g. with a radioactive tag. The hybridization reaction could be carried out in a solution comprising 2X SSC and 0% formamide, at a temperature of about 37°C (low stringency conditions). Solutions of differing concentrations of SSC can be made by one of skill in the art by diluting a stock solution of 20X SSC (175.3 gram NaCl and about 88.2 gram sodium citrate in 1 liter of water, pH 7) to obtain the desired concentration of SSC. In order to achieve high stringency hybridization, the skilled artisan would calculate the washing conditions required to allow up to 30% base-pair mismatch. For example, in a wash solution comprising 1X SSC and 0% formamide, the T_m of perfect hybrids would be about 80.8°C:

$$81.5^{\circ}\text{C} + 16.6 \log (.15\text{M}) + (0.41 \times 40) - (500/150) - (0.61 \times 0) = 80.8^{\circ}\text{C}.$$

Thus, to achieve hybridization with nucleic acid molecules having about 30% base-pair mismatch, hybridization washes would be carried out at a temperature of about 50.8°C.

- It is within the skill of one in the art to calculate the hybridization temperature based on the formulae and G/C content disclosed herein.

Furthermore, it is known in the art that there are commercially available computer programs for determining the degree of similarity between two nucleic acid sequences. These computer programs include various known methods to determine the percentage identity and the number and length of gaps between hybrid nucleic acid molecules. Preferred methods to determine the percent identity among amino acid sequences and also among nucleic acid sequences include analysis using one or more of the commercially available computer programs designed to compare and analyze nucleic acid or amino acid sequences. These computer programs include, but are not limited to, the Wisconsin Package Version 9.0 sequence analysis software, available from Genetics Computer Group (GCG), Madison, WI; DNAsis™, available from Hitachi Software, San Bruno, CA; and MacVector™, available from the Eastman Kodak Company, New Haven, CT. A preferred method to determine percent identity among amino acid sequences and also among nucleic acid sequences includes using the GAP program with pair-wise comparisons within the Wisconsin Package Version 9.0 sequence analysis software, available from Genetics Computer Group (GCG), Madison, WI, hereinafter referred to as default parameters.

The present invention also includes mimetopes of B7 and CTLA4 proteins of the present invention. As used herein, a mimetope of a B7 or CTLA4 protein of the present invention refers to any compound that is able to mimic the activity of such a B7 or CTLA4 protein, often because the mimetope has a structure that mimics the particular B7 or CTLA4 protein. Mimetopes can be, but are not limited to: peptides that have been modified to decrease their susceptibility to degradation such as all-D retro peptides; anti-idiotypic and/or catalytic antibodies, or fragments thereof; non-proteinaceous immunogenic portions of an isolated protein (e.g., carbohydrate structures); and synthetic or natural organic molecules, including nucleic acids. Such mimetopes can be designed using computer-generated structures of proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides or other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner.

One embodiment of a B7 or CTLA4 protein of the present invention is a fusion protein that includes a B7 or CTLA4 protein-containing domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but are not limited to, segments that can: link two or more B7 or CTLA4 proteins to form multimeric forms of B7 or CTLA4 protein; enhance a protein's stability; act as an immunopotentiator to enhance an immune response against a B7 or CTLA4 protein; and/or assist in purification of a B7 or CTLA4 protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired function (e.g., imparts increased stability, imparts increased immunogenicity to a protein, and/or simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of the B7 or CTLA4-containing domain of the protein and can be susceptible to cleavage in order to enable straight-forward recovery of a B7 or CTLA4 protein. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid molecule that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of a B7 or CTLA4-containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; Fc receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); and/or a "tag" domain (e.g., at least a portion of β -galactosidase, a strep tag peptide, a T7 tag peptide, a FlagTM peptide, or other domains that can be purified using compounds that bind to the domain, such as monoclonal antibodies). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding domain; a strep tag peptide, such as that available from Biometra in Tampa, FL; and an S10 peptide.

A suitable fusion segment that links one B7 or CTLA4 protein to another B7 or CTLA4 protein includes any amino acid sequence that enables B7 or CTLA4 proteins to be linked while maintaining the biological function of the B7 or CTLA4 proteins. Selection of a suitable linker is dependent upon how many B7 or CTLA4 proteins are to be linked to form one multimeric molecule and from where on the B7 or CTLA4 molecule the linker extends. Preferably, a linker fusion segment of the present invention

comprises a peptide of from about 6 amino acid residues to about 40 residues, more preferably from about 6 residues to about 30 residues in length.

In another embodiment, a B7 or CTLA4 protein of the present invention also includes at least one additional protein segment that is capable of targeting a B7 or CTLA4 protein to a desired cell or receptive molecule. Such a multivalent protective protein can be produced by culturing a cell transformed with a nucleic acid molecule comprising two or more nucleic acid domains joined together in such a manner that the resulting nucleic acid molecule is expressed as a multivalent targeting protein containing a B7 or CTLA4 protein or portion thereof and at least one targeting compound capable of delivering the B7 or CTLA4 protein to a desired site in an animal.

Examples of multivalent targeting proteins include, but are not limited to, a B7 or CTLA4 protein of the present invention attached to one or more compounds that can bind to a receptive molecule on the surface of a cell located in an area of an animal where regulation of an immune response is desired. One of skill in the art can select appropriate targeting fusion segments depending upon the cell or receptive molecule being targeted.

A naturally-occurring variant of B7-1 or B7-2 protein of the present invention is preferably isolated from (including isolation of the natural protein or production of the protein by recombinant or synthetic techniques) from mammals, including but not limited to dogs (i.e., canids), cats (i.e., felids), horses (i.e., equids), humans, cattle, chinchillas, ferrets, goats, mice, minks, rabbits, raccoons, rats, sheep, squirrels, swine, chickens, ostriches, quail and turkeys as well as other furry animals, pets, zoo animals, work animals and/or food animals. Particularly preferred animals from which to isolate B7 and CTLA4 proteins are dogs, cats, horses and humans.

A preferred isolated protein of the present invention is a protein encoded by at least one of the following nucleic acid molecules: nCaB7-1₂₈₃₀, nCaB7-1₁₃₈₅, nCaB7-1₉₁₂, nCaB7-2₁₈₉₇, nCaB7-2₉₈₇, nCaB7-1s₁₀₂₄, nCaB7-1s₇₀₅, nCaB7-2s₁₇₉₅, nCaB7-2s₈₄₀, nFeB7-2₁₈₉₇, nFeB7-2₉₉₆, λ -nCaB7-2₁₈₉₇, pCMV-nCaB7-2₁₈₉₇, λ -nCaB7-1₂₈₃₀, pCMV-nCaB7-1₁₃₈₅, λ -nFeB7-2₁₈₉₇, nCaB7-1₈₁₀, nCaB7-2₉₂₁, nCaB7-1s₆₀₃, nCaB7-2s₇₇₄ and/or nFeB7-2₉₁₈, or allelic variants of any of these nucleic acid molecules. Also preferred is an isolated protein that is encoded by a nucleic acid molecule the having nucleic acid

sequence SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:25 and/or SEQ ID NO:28; or an allelic variant of such a nucleic acid molecule.

Translation of SEQ ID NO:1, the coding strand of nCaB7-1₂₈₃₀, yields a protein
 5 of about 304 amino acids, denoted herein as PCaB7-1₃₀₄, the amino acid sequence of which is presented in SEQ ID NO:2, assuming an open reading frame having an initiation codon spanning from nucleotide 337 through nucleotide 339 of SEQ ID NO:1 and a stop codon spanning from nucleotide 1249 through nucleotide 1251 of SEQ ID NO:1. The coding region encoding PCaB7-1₃₀₄ is presented herein as nCaB7-1₉₁₂, which
 10 has the nucleotide sequence SEQ ID NO:4 (the coding strand) and SEQ ID NO:5 (the complementary strand).

Translation of SEQ ID NO:6, the coding strand of nCaB7-2₁₈₉₇, yields a protein of about 329 amino acids, denoted herein as PCaB7-2₃₂₉, the amino acid sequence of which is presented in SEQ ID NO:7, assuming an open reading frame having an
 15 initiation codon spanning from nucleotide 6 through nucleotide 8 of SEQ ID NO:6 and a stop codon spanning from nucleotide 993 through nucleotide 995 of SEQ ID NO:6. The coding region encoding PCaB7-2₃₂₉, not including the termination codon, is presented herein as nCaB7-2₉₈₇, which has the nucleotide sequence SEQ ID NO:9 (the coding strand) and SEQ ID NO:10 (the complementary strand).

20 Translation of SEQ ID NO:25, the coding strand of nFeB7-2₁₈₉₇, yields a protein of about 332 amino acids, denoted herein as PFeB7-2₃₂₉, the amino acid sequence of which is presented in SEQ ID NO:26, assuming an open reading frame having an initiation codon spanning from nucleotide 6 through nucleotide 8 of SEQ ID NO:25 and a stop codon spanning from nucleotide 993 through nucleotide 995 of SEQ ID NO:25.
 25 The coding region encoding PFeB7-2₃₂₉ is presented herein as nFeB7-2₉₉₆, which has the nucleotide sequence SEQ ID NO:28 (the coding strand) and SEQ ID NO:29 (the complementary strand).

One embodiment of a B7 or CTLA4 protein of the present invention includes a naturally-occurring variant of B7 or CTLA4 protein. As used herein, a naturally-
 30 occurring variant refers to a B7 or CTLA4 protein that is originally encoded by a nucleic acid molecule that, in its native form, i.e., a native sequence encoded by a gene, and not

mutated by human manipulation such as by recombinant or synthetic techniques that, for example, mutate, modify or create a variant B7 nucleic acid sequence, encodes a B7 variant. According to the present invention, copies of nucleic acid molecules encoding naturally-occurring variants can be produced recombinantly. Without being bound by theory, a variant may arise due to DNA recombination, alternative RNA splicing, or a mutation resulting in premature termination of translation. Examples include soluble B7 or CTLA4 proteins lacking at least a portion of transmembrane domain, or all or a portion of the transmembrane and intracellular domains, sufficient to produce a soluble protein upon translation of a nucleic acid molecule encoding the protein, either *in vitro* or in a suitable host cell. Such a protein retains the ability to bind to CD28 and/or CTLA4, and stimulate T cell activity, but is soluble. Preferred B7 variants include B7-1 and B7-2 variants. One example of a naturally-occurring B7-1 variant is a B7-1 protein encoded by a nucleic acid molecule lacking at least a portion of the nucleic acid molecule that encodes the transmembrane and intracellular domains of the B7-1 protein, sufficient to produce a soluble B7-1 protein upon translation of a nucleic acid molecule encoding the protein, either *in vivo* or in a suitable host cell. Another example of a naturally-occurring B7-2 variant is a B7-2 protein encoded by a nucleic acid molecule lacking at least a portion of the nucleic acid molecule that encodes the transmembrane domain, sufficient to produce a soluble B7-2 protein upon translation of a nucleic acid molecule encoding the protein, either *in vivo* or in a suitable host cell. A naturally-occurring B7-1 variant of the present invention is preferably encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a nucleic acid molecule including SEQ ID NO:13, and/or SEQ ID NO:15. More preferably, a naturally-occurring B7-1 variant comprises an amino acid molecule including SEQ ID NO:12. More preferably, a naturally-occurring B7-1 variant of the present invention is encoded by a nucleic acid molecule that comprises a nucleic acid molecule including SEQ ID NO:11, and/or SEQ ID NO:14.

An example of a naturally-occurring B7-1 variant is encoded by the nucleic acid molecule nCaB7-1_{S1024}. Translation of SEQ ID NO:11, the coding strand of nCaB7-1_{S1024}, yields a protein of about 235 amino acids, denoted herein as PCaB7-1_{S235}, the amino acid sequence of which is presented in SEQ ID NO:12, assuming an open reading

frame having an initiation codon spanning from nucleotide 79 through nucleotide 81 of SEQ ID NO:11 and a stop codon spanning from nucleotide 784 through nucleotide 786 of SEQ ID NO:11. The coding region encoding PCaB7-1s₂₃₅, not including the termination codon, is presented herein as nCaB7-1s₇₀₅, which has the nucleotide
5 sequence SEQ ID NO:14 (the coding strand) and SEQ ID NO:15 (the complementary strand).

One example of a naturally-occurring B7-2 variant is a B7-2 protein lacking at least a portion of the transmembrane domain. Such a naturally-occurring B7-2 variant is preferably encoded by a nucleic acid molecule that hybridizes under stringent
10 hybridization conditions to a nucleic acid molecule including SEQ ID NO:18, and/or SEQ ID NO:20. More preferably, a naturally-occurring of B7-2 variant comprises an amino acid molecule including SEQ ID NO:17. More preferably, a naturally-occurring of B7-2 variant is encoded by a nucleic acid molecule that comprises a nucleic acid molecule including SEQ ID NO:16, and/or SEQ ID NO:19.

15 An example of a naturally-occurring B7-2 variant is encoded by the nucleic acid molecule nCaB7-2s₁₇₉₅. Translation of SEQ ID NO:16, the coding strand of nCaB7-2s₁₇₉₅, yields a protein of about 280 amino acids, denoted herein as PCaB7-2s₂₈₀, the amino acid sequence of which is presented in SEQ ID NO:17, assuming an open reading frame having an initiation codon spanning from nucleotide 7 through nucleotide 9 of
20 SEQ ID NO:16 and a stop codon spanning from nucleotide 847 through nucleotide 849 of SEQ ID NO:16. The coding region encoding PCaB7-2s₂₈₀, not including the termination codon, is presented herein as nCaB7-2s₈₄₀, which has the nucleotide sequence SEQ ID NO:19 (the coding strand) and SEQ ID NO:20 (the complementary strand). SEQ ID NO:16 is predicted to encode a B7-2 protein lacking at least a portion
25 of the transmembrane domain.

Preferred B7 proteins of the present invention include proteins that are at least about 80%, preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to PCaB7-1₃₀₄, or epitopes thereof; are
30 more preferably at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, and even more preferably at least

about 95%, identical to PCaB7-2₃₂₉, or epitopes thereof; are at least about 80%, preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to PCaB7-1s₂₃₅, or epitopes thereof; are at least about 60%, preferably at least about 65%, more preferably at least about 70%, more preferably at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, and even more preferably at least about 95%, identical to PCaB7-2s₂₈₀, or epitopes thereof; or are at least about 60%, preferably at least about 65%, more preferably at least about 70%, more preferably at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, and even more preferably at least about 95%, identical to PFeB7-2₃₃₂, or epitopes thereof.

More preferred are B7 proteins comprising PCaB7-1₃₀₄, PCaB7-2₃₂₉, PCaB7-1s₂₃₅, PCaB7-2s₂₈₀, PFeB7-2₃₃₂, PCaB7-1₂₇₀, PCaB7-2₃₀₇, PCaB7-1s₂₀₁, PCaB7-2s₂₅₈ or PFeB7-2₃₀₉, and proteins encoded by allelic variants of nucleic acid molecules encoding proteins PCaB7-1₃₀₄, PCaB7-2₃₂₉, PCaB7-1s₂₃₅, PCaB7-2s₂₈₀, PFeB7-2₃₃₂, PCaB7-1₂₇₀, PCaB7-2₃₀₇, PCaB7-1s₂₀₁, PCaB7-2s₂₅₈ or PFeB7-2₃₀₉.

Also preferred are B7 proteins of the present invention having amino acid sequences that are at least about 80%, preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:2, or epitopes thereof; are at least about 60%, preferably at least about 65%, more preferably at least about 70%, more preferably at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, and even more preferably at least about 95%, identical to SEQ ID NO:7, or epitopes thereof; are at least about 80%, preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to, identical to SEQ ID NO:12, or epitopes thereof; are at least about 60%, preferably at least about 65%, more preferably at least about 70%, more preferably at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, and even more preferably at least about 95%, identical to SEQ ID NO:17, or epitopes thereof; or are at least about 60%, preferably at least about 65%, more preferably at least about 70%, more preferably at least about 75%, more preferably

at least about 80%, more preferably at least about 85%, more preferably at least about 90%, and even more preferably at least about 95%, identical to SEQ ID NO:26, or epitopes thereof. More preferred are B7 proteins comprising amino acid sequences SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12, SEQ ID NO:17 and/or SEQ ID NO:26; and B7
5 proteins encoded by allelic variants of nucleic acid molecules encoding B7 proteins having amino acid sequences SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12, SEQ ID NO:17 and/or SEQ ID NO:26.

Preferred CTLA4 proteins of the present invention include proteins that are at least about 90%, preferably at least about 95% identical to PCaCTLA4₂₂₄, or epitopes
10 thereof; or are at least about 90%, preferably at least about 95% identical to PFeCTLA4₂₂₃, or epitopes thereof. More preferred are CTLA4 proteins comprising PCaCTLA4₂₂₃, or PFeCTLA4₂₂₃, and proteins encoded by allelic variants of nucleic acid molecules encoding proteins PCaCTLA4₂₂₃, or PFeCTLA4₂₂₃.

Also preferred are CTLA4 proteins of the present invention having amino acid
15 sequences that are at least about 90%, preferably at least about 95% identical to SEQ ID NO:41, or epitopes thereof; or are at least about 90%, preferably at least about 95% identical to SEQ ID NO:46, or epitopes thereof. More preferred are CTLA4 proteins comprising amino acid sequences SEQ ID NO:41, and/or SEQ ID NO:46; and CTLA4 proteins encoded by allelic variants of nucleic acid molecules encoding CTLA4 proteins
20 having amino acid sequences SEQ ID NO:41, and/or SEQ ID NO:46.

Percent identities between amino acid or nucleic acid sequences can be determined using standard methods known to those of skill in the art. It is known in the art that methods to determine the percentage identity and the number of gaps are substantially similar when different methods for determining sequence similarity are
25 used and when the degree of similarity is greater than 30% amino acid identity, as described by Johnson et al., *J. Mol. Biol.*, vol. 233, pages 716-738, 1993, and Feng et al., *J. Mol. Evol.*, vol. 21, pages 112-125, 1985. Preferred methods to determine percentage identities between amino acid sequences and between nucleic acid sequences include comparison using various computer programs such as the Wisconsin Package Version
30 9.0 sequence analysis software, available from Genetics Computer Group (GCG), Madison, WI; DNAsis™ program, available from Hitachi Software, San Bruno, CA; or

the MacVector™ program, available from the Eastman Kodak Company, New Haven, CT. A preferred method to determine percentage identities between amino acid sequences and between nucleic acid sequences includes using the DNAsis™ computer program with the following settings: the gap penalty set at 5; the number of top
 5 diagonals set at 5; the fixed gap penalty set at 10; the k-tuple set at 2; the window size set at 5 and the floating gap penalty set at 10.

Additional preferred B7 or CTLA4 proteins of the present invention include proteins encoded by nucleic acid molecules comprising at least a portion of nCaB7-1₂₈₃₀, nCaB7-1₁₃₈₅, nCaB7-1₉₁₂, nCaB7-2₁₈₉₇, nCaB7-2₉₈₇, nCaB7-1s₁₀₂₄, nCaB7-1s₇₀₅, nCaB7-
 10 2s₁₇₉₅, nCaB7-2s₈₄₀, nFeB7-2₂₈₃₀, nFeB7-2₉₉₆, nCaB7-1₈₁₀, nCaB7-2₉₂₁, nCaB7-1s₆₀₃, nCaB7-2s₇₇₄, nFeB7-2₉₁₈, nFeB7-2₅₀₉, nFeB7-2s₃₅₉, nFeB7-1s₅₉₄, nFeB7-1s₅₁₉, nCaCTLA4₁₈₅₆, nCaCTLA4₆₇₂, nFeCTLA4₁₈₈₃, and/or nFeCTLA₄₆₇₂, as well as B7 or CTLA4 proteins encoded by allelic variants of such nucleic acid molecules.

Also preferred are B7 proteins encoded by nucleic acid molecules having nucleic
 15 acid sequences comprising at least a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID
 20 NO:35, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, and/or SEQ ID NO:50, as well as allelic variants of these nucleic acid molecules.

Another embodiment of the present invention is a B7 or CTLA4 nucleic acid
 25 molecule that includes one or more regulatory regions, full-length or partial coding regions, or combinations thereof. The minimal size of a nucleic acid molecule of the present invention is a size sufficient to allow the formation of a stable hybrid (i.e., hybridization under stringent hybridization conditions) with the complementary sequence of another nucleic acid molecule. As such, the minimal size of a B7 or CTLA4
 30 nucleic acid molecule of the present invention is from about 12 to about 18 nucleotides in length.

In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subjected to human manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated B7 or CTLA4 nucleic acid molecule of the present invention can be isolated from its natural source or produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification or cloning) or chemical synthesis. Isolated B7 or CTLA4 nucleic acid molecules can include, for example, natural allelic variants and nucleic acid molecules modified by nucleotide insertions, deletions, substitutions, and/or inversions in a manner such that the modifications do not substantially interfere with the nucleic acid molecule's ability to encode a B7 protein of the present invention.

A B7 or CTLA4 nucleic acid molecule homolog can be produced using a number of methods known to those skilled in the art, see, for example, Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press. For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis and recombinant DNA techniques such as site-directed mutagenesis, chemical treatment, restriction enzyme cleavage, ligation of nucleic acid fragments, PCR amplification, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules, and combinations thereof. Nucleic acid molecule homologs can be selected by hybridization with a B7 or CTLA4 nucleic acid molecule or by screening the function of a protein encoded by the nucleic acid molecule (e.g., ability to elicit an immune response against at least one epitope of a B7 protein).

An isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one B7 protein of the present invention, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a B7 protein.

A preferred nucleic acid molecule of the present invention, when administered to an animal, is capable of regulating an immune response in an animal. As will be disclosed in more detail below, such a nucleic acid molecule can be, or encode, an antisense RNA, a molecule capable of triple helix formation, a ribozyme, or other nucleic acid-based drug compound. In additional embodiments, a nucleic acid molecule of the present invention can encode an immunoregulatory protein (e.g., a cell-bound or soluble B7 protein of the present invention), the nucleic acid molecule being delivered to the animal, for example, by direct injection (i.e., as a genetic vaccine) or in a vehicle such as a recombinant virus vaccine or a recombinant cell vaccine.

- One embodiment of the present invention is a B7 or CTLA4 nucleic acid molecule comprising all or part (i.e., a fragment of the B7 or CTLA4 nucleic acid molecule) of nucleic acid molecules nCaB7-1₂₈₃₀, nCaB7-1₁₃₈₅, nCaB7-1₉₁₂, nCaB7-2₁₈₉₇, nCaB7-2₉₈₇, nCaB7-1s₁₀₂₄, nCaB7-1s₇₀₅, nCaB7-2s₁₇₉₅, nCaB7-2s₈₄₀, nFeB7-2₂₈₃₀, nFeB7-2₉₉₆, nCaB7-1₈₁₀, nCaB7-2₉₂₁, nCaB7-1s₆₀₃, nCaB7-2s₇₇₄, nFeB7-2₉₁₈, nFeB7-2₅₀₉, nFeB7-2s₃₅₉, nFeB7-1s₅₉₄, nFeB7-1s₅₁₉, nCaCTLA4₁₈₅₆, nCaCTLA4₆₇₂, nFeCTLA4₁₈₈₃, and/or nFeCTLA₄₆₇₂, or allelic variants of these nucleic acid molecules. Another preferred nucleic acid molecule of the present invention includes at least a portion of (i.e., a fragment of the nucleic acid molecule) nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, and/or SEQ ID NO:50, as well as allelic variants of nucleic acid molecules having these nucleic acid sequences. Such nucleic acid molecules can include nucleotides in addition to those included in the SEQ ID NOs, such as, but not limited to, a full-length gene, a full-length coding region, a nucleic acid molecule encoding a fusion protein, or a nucleic acid molecule encoding a multivalent therapeutic compound.

In one embodiment, a B7 nucleic acid molecule of the present invention encodes a protein that is at least about 80%, preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to PcaB7-1₃₀₄ or PCaB7-1s₂₃₅; or are at least about 60%, preferably at least about 65%, more preferably at least about 70%, more preferably at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, and even more preferably at least about 95%, identical to PCaB7-2₃₂₉, PCaB7-2s₂₈₀, or PFeB7-2₃₃₂. Even more preferred is nucleic acid molecule encoding PCaB7-1₃₀₄, PCaB7-2₃₂₉, PCaB7-1s₂₃₅, PCaB7-2s₂₈₀, PFeB7-2₃₃₂, PFeB7-2₁₆₉, PFeB7-2₁₁₉, PCaB7-1₂₇₀, PCaB7-2₃₀₇, PCaB7-1s₂₀₁, PCaB7-2s₂₅₈, or PFeB7-2₃₀₉, and/or an allelic variant of such a nucleic acid molecule.

In another embodiment, a B7 nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least about 80%, preferably at least about 85%, even more preferably at least about 90%, and even more preferably at least about 95% identical to SEQ ID NO:2 or SEQ ID NO:12; or at least about 60%, preferably at least about 65%, more preferably at least about 70%, more preferably at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, and even more preferably at least about 95%, identical to SEQ ID NO:7, SEQ ID NO:17 or SEQ ID NO:26. The present invention also includes a B7 nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12, SEQ ID NO:17, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:37, as well as allelic variants of a B7 nucleic acid molecule encoding a protein having these sequences, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In one embodiment, a B7 nucleic acid molecule of the present invention is at least about 80%, preferably at least about 85%, more preferably at least about 90%, and more preferably at least about 95% identical to nCaB7-1₂₈₃₀, nCaB7-2₁₈₉₇, nCaB7-1s₁₀₂₄, nCaB7-2₁₈₉₇, nCaB7-2s₁₇₉₅, nCaB7-1s₁₀₂₄, or nFeB7-2₁₈₉₇. Even more preferred is a nucleic acid molecule comprising nCaB7-1₂₈₃₀, nCaB7-1₉₁₂, nCaB7-2₁₈₉₇, nCaB7-2₉₈₇, nCaB7-1s₁₀₂₄, nCaB7-1s₇₀₅, nCaB7-2s₁₇₉₅, nCaB7-2s₈₄₀, nFeB7-2₁₈₉₇, nFeB7-2₉₉₆, nCaB7-

1₈₁₀, nCaB7-2₉₂₁, nCaB7-1s₆₀₃, nCaB7-2s₇₇₄, nFeB7-2₉₁₈, and/or an allelic variant of such a nucleic acid molecule.

In another embodiment, a B7 nucleic acid molecule of the present invention comprises a nucleic acid sequence that is at least about 80%, preferably at least about 85%, more preferably at least about 90%, and more preferably at least about 95% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, or SEQ ID NO:40. The present invention also includes a B7 nucleic acid molecule comprising at least a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, and/or SEQ ID NO:40 as well as allelic variants of such B7 nucleic acid molecules, including nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In one embodiment, a CTLA4 nucleic acid molecule of the present invention encodes a protein that is at least about 90%, and preferably at least about 95% identical to PCaCTLA4₂₂₃ or PFeCTLA4₂₂₃. Even more preferred is nucleic acid molecule encoding PCaCTLA4₂₂₃ or PFeCTLA4₂₂₃, and/or an allelic variant of such a nucleic acid molecule.

In another embodiment, a CTLA4 nucleic acid molecule of the present invention encodes a protein having an amino acid sequence that is at least about 90%, and preferably at least about 95% identical to SEQ ID NO:42 or SEQ ID NO:47. The present invention also includes a CTLA4 nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:42, SEQ ID NO:46, as well as allelic variants of a CTL4 nucleic acid molecule encoding a protein having these sequences, including

nucleic acid molecules that have been modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

In one embodiment, a CTLA4 nucleic acid molecule of the present invention is at least about 90%, and preferably at least about 95% identical to nCaCTLA4₁₈₅₆ or
5 nFeCTLA4₁₈₈₃. Even more preferred is a nucleic acid molecule comprising nCaCTLA4₁₈₅₆, nFeCTLA4₁₈₈₃, and/or an allelic variant of such a nucleic acid molecule.

In another embodiment, a CTLA4 nucleic acid molecule of the present invention comprises a nucleic acid sequence that is at least about 90%, and preferably at least about 95% identical to SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45,
10 SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, or SEQ ID NO:50. The present invention also includes a CTLA4 nucleic acid molecule comprising at least a portion of SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, and/or SEQ ID NO:50, as well as allelic variants of such CTLA4 nucleic acid molecules, including nucleic acid molecules that have been
15 modified to accommodate codon usage properties of the cells in which such nucleic acid molecules are to be expressed.

Knowing the nucleic acid sequences of certain B7 and CTLA4 nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules, (b) obtain nucleic acid molecules including at
20 least a portion of such nucleic acid molecules (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and (c) obtain other B7 or CTLA4 nucleic acid molecules. Such nucleic acid molecules can be obtained in a variety of ways including screening appropriate expression libraries with antibodies of the present invention; traditional
25 cloning techniques using oligonucleotide probes of the present invention to screen appropriate libraries; and PCR amplification of appropriate libraries or DNA using oligonucleotide primers of the present invention. Preferred libraries to screen or from which to amplify nucleic acid molecules include mammalian cDNA libraries as well as genomic DNA libraries. Similarly, preferred DNA sources from which to amplify
30 nucleic acid molecules include mammalian cDNA and genomic DNA. Techniques to clone and amplify genes are disclosed, for example, in Sambrook et al., *ibid*.

The present invention also includes nucleic acid molecules that are oligonucleotides capable of hybridizing, under stringent hybridization conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising B7 or CTLA4 nucleic acid molecules.

- 5 Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of such oligonucleotides is the size required for formation of a stable hybrid between an oligonucleotide and a complementary sequence on a nucleic acid molecule of the present invention. A preferred oligonucleotide of the present invention has a maximum size of preferably about 200 nucleotides, more preferably about 150
- 10 nucleotides, more preferably about 100 nucleotides and even more preferably about 50 nucleotides. The present invention includes oligonucleotides that can be used as, for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules, or therapeutic reagents to inhibit B7 or CTLA4 protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The
- 15 present invention also includes the use of such oligonucleotides to protect animals from disease using one or more of such technologies. Appropriate oligonucleotide-containing therapeutic compositions can be administered to an animal using techniques known to those skilled in the art.

- One embodiment of the present invention includes a recombinant vector, which
- 20 includes at least one isolated nucleic acid molecule of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to nucleic acid molecules of the present invention and that preferably are derived from a species other than the species from which the nucleic acid
- 25 molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulating of B7 or CTLA4 nucleic acid molecules of the present invention.

- One type of recombinant vector, referred to herein as a recombinant molecule,
- 30 comprises a nucleic acid molecule of the present invention operatively linked to an expression vector. The phrase operatively linked refers to insertion of a nucleic acid

molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also
5 capable of replicating within the host cell. Expression vectors can be either prokaryotic or eukaryotic, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in recombinant cells of the present invention, including in bacterial, fungal, parasite, insect, other animal, and plant cells. Preferred expression vectors of the present invention can direct
10 gene expression in bacterial, yeast, insect and mammalian cells, and more preferably in the cell types disclosed herein, more preferably *in vivo*.

In particular, expression vectors of the present invention contain regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant
15 cell and that control the expression of nucleic acid molecules of the present invention. In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as
20 promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in bacterial, yeast, helminth or other
25 endoparasite, or insect and mammalian cells, such as, but not limited to, *tac*, *lac*, *trp*, *trc*, *oxy-pro*, *omp/lpp*, *rmb*, bacteriophage lambda (such as lambda p_L and lambda p_R and fusions that include such promoters), bacteriophage T7, T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha-mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoter, antibiotic resistance gene,
30 baculovirus, *Heliothis zea* insect virus, vaccinia virus, herpesvirus, raccoon poxvirus, other poxvirus, adenovirus, cytomegalovirus (such as immediate early promoter), simian

virus 40, retrovirus, actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control sequences as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by interferons or interleukins). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with mammals, such as dog, cat, horse, or human transcription control sequences.

Suitable and preferred nucleic acid molecules to include in recombinant vectors of the present invention are as disclosed herein. Preferred nucleic acid molecules to include in recombinant vectors, and particularly in recombinant molecules, include nCaB7-1₂₈₃₀, nCaB7-1₁₃₈₅, nCaB7-1₉₁₂, nCaB7-2₁₈₉₇, nCaB7-2₉₈₇, nCaB7-1s₁₀₂₄, nCaB7-1s₇₀₅, nCaB7-2s₁₇₉₅, nCaB7-2s₈₄₀, nFeB7-2₂₈₃₀, nFeB7-2₉₉₆, nCaB7-1₈₁₀, nCaB7-2₉₂₁, nCaB7-1s₆₀₃, nCaB7-2s₇₇₄, nFeB7-2₉₁₈, nFeB7-2₅₀₉, nFeB7-2s₃₅₉, nFeB7-1s₅₉₄, nFeB7-1s₅₁₉, nCaCTLA4₁₈₅₆, nCaCTLA4₆₇₂, nFeCTLA4₁₈₈₃, and nFeCTLA4₄₆₇₂.

Recombinant molecules of the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed parasitic helminth protein of the present invention to be secreted from the cell that produces the protein and/or (b) contain fusion sequences which lead to the expression of nucleic acid molecules of the present invention as fusion proteins. Examples of suitable signal segments include any signal segment capable of directing the secretion of a protein of the present invention. Preferred signal segments include, but are not limited to, tissue plasminogen activator (t-PA), interferon, interleukin, growth hormone, histocompatibility and viral envelope glycoprotein signal segments. Suitable fusion segments encoded by fusion segment nucleic acids are disclosed herein. In addition, a nucleic acid molecule of the present invention can be joined to a fusion segment that directs the encoded protein to the proteosome, such as a ubiquitin fusion segment. Eukaryotic recombinant molecules may also include intervening and/or untranslated sequences surrounding and/or within the nucleic acid sequences of nucleic acid molecules of the present invention. Preferred recombinant molecules of the present

invention include λ -nCaB7-2₁₈₉₇, pCMV-nCaB7-2₁₈₉₇, λ -nCaB7-1₂₈₃₀, pCMV-nCaB7-1₁₃₈₅ or λ -nFeB7-2₁₈₉₇.

- Another embodiment of the present invention includes a recombinant cell comprising a host cell transformed with one or more recombinant molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include B7 or CTLA4 nucleic acid molecules disclosed herein.
- Particularly preferred nucleic acid molecules with which to transform a cell include nCaB7-1₂₈₃₀, nCaB7-1₁₃₈₅, nCaB7-1₉₁₂, nCaB7-2₁₈₉₇, nCaB7-2₉₈₇, nCaB7-1s₁₀₂₄, nCaB7-1s₇₀₅, nCaB7-2s₁₇₉₅, nCaB7-2s₈₄₀, nFeB7-2₂₈₃₀, nFeB7-2₉₉₆, nCaB7-1₈₁₀, nCaB7-2₉₂₁, nCaB7-1s₆₀₃, nCaB7-2s₇₇₄, nFeB7-2₉₁₈, nFeB7-2₅₀₉, nFeB7-2s₃₅₉, nFeB7-1s₅₉₄, nFeB7-1s₅₁₉, nCaCTLA4₁₈₅₆, nCaCTLA4₆₇₂, nFeCTLA4₁₈₈₃, and nFeCTLA4₄₆₇₂.
- Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule (e.g., nucleic acid molecules encoding one or more proteins of the present invention and/or other proteins useful in the production of multivalent vaccines). Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing B7 or CTLA4 proteins of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal (including yeast), parasite (including helminth, protozoa and ectoparasite), other insect, other animal and plant cells. Preferred host cells include bacterial, mycobacterial, yeast, helminth, insect and

mammalian cells. More preferred host cells include *Salmonella*, *Escherichia*, *Bacillus*, *Listeria*, *Saccharomyces*, *Spodoptera*, *Mycobacteria*, *Trichoplusia*, BHK (baby hamster kidney) cells, MDCK cells (Madin-Darby canine kidney cell line), CRFK cells (Crandell feline kidney cell line), CV-1 cells (African monkey kidney cell line used, for example, to culture raccoon poxvirus), COS (e.g., COS-7) cells, chinese hamster ovary (CHO) cells, Ltk cells and Vero cells. Particularly preferred host cells are *Escherichia coli*, including *E. coli* K-12 derivatives; *Salmonella typhi*; *Salmonella typhimurium*, including attenuated strains such as UK-1₀₃₉₈₇ and SR-11₀₄₀₇₂; *Spodoptera frugiperda*; *Trichoplusia ni*; BHK cells; MDCK cells; CRFK cells; CV-1 cells; COS cells; Vero cells; and non-tumorigenic mouse myoblast G8 cells (e.g., ATCC CRL 1246).

Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., human, murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK³¹ cells and/or HeLa cells. In one embodiment, the proteins may be expressed as heterologous proteins in myeloma cell lines employing immunoglobulin promoters.

A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules of the present invention operatively linked to an expression vector containing one or more transcription control sequences, examples of which are disclosed herein.

A recombinant cell of the present invention includes any cell transformed with at least one of any nucleic acid molecule of the present invention. Suitable and preferred nucleic acid molecules as well as suitable and preferred recombinant molecules with which to transfer cells are disclosed herein.

Recombinant cells of the present invention can also be co-transformed with one or more recombinant molecules including B7 or CTLA4 nucleic acid molecules encoding one or more proteins of the present invention and one or more other nucleic acid molecules encoding other protective compounds, as disclosed herein (e.g., to produce multivalent vaccines).

Preferred recombinant cells of the present invention include CHO-pCMV-nCaB7-2₁₈₉₇ or CHO-pCMV-nCaB7-1₁₃₈₅.

Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

Isolated B7 or CTLA4 proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins, production and recovery of recombinant proteins, and chemical synthesis of the proteins. In one embodiment, an isolated protein of the present invention is produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell of the present invention. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective medium refers to any medium in which a cell is cultured to produce a B7 or CTLA4 protein of the present invention. Such medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature,

pH and oxygen content appropriate for a recombinant cell. Such culturing conditions are within the expertise of one of ordinary skill in the art.

Depending on the vector and host system used for production, resultant proteins of the present invention may either remain within the recombinant cell; be secreted into
5 the fermentation medium; be secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or be retained on the outer surface of a cell or viral membrane.

The phrase "recovering the protein", as well as similar phrases, refers to collecting the whole fermentation medium containing the protein and need not imply
10 additional steps of separation or purification. Proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and
15 differential solubilization. Proteins of the present invention are preferably retrieved in "substantially pure" form. As used herein, "substantially pure" refers to a purity that allows for the effective use of the protein as a therapeutic composition or diagnostic. A therapeutic composition for animals, for example, should exhibit no substantial toxicity and preferably should be capable of stimulating the production of antibodies in a treated
20 animal.

The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to a B7 or CTLA4 protein of the present invention or a mimetope thereof (e.g., anti-B7 or CTLA4 antibodies). As used herein, the term "selectively binds to" a B7 or CTLA4 protein refers to the ability of antibodies
25 of the present invention to preferentially bind to specified proteins and mimetopes thereof of the present invention. Binding can be measured using a variety of methods standard in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., *ibid.*, and Harlow, et al., 1988, *Antibodies, a Laboratory Manual*, Cold Spring Harbor Labs Press. An anti-B7 or CTLA4 antibody of
30 the present invention preferably selectively binds to a B7 or CTLA4 protein in such a way as to inhibit the function of that protein.

Isolated antibodies of the present invention can include antibodies in serum, or antibodies that have been purified to varying degrees. Antibodies of the present invention can be polyclonal or monoclonal, or can be functional equivalents such as antibody fragments and genetically-engineered antibodies, including single chain
5 antibodies or chimeric antibodies that can bind to one or more epitopes.

A preferred method to produce antibodies of the present invention includes (a) administering to an animal an effective amount of a protein, peptide or mimetope thereof of the present invention to produce the antibodies and (b) recovering the antibodies. In another method, antibodies of the present invention are produced
10 recombinantly using techniques as heretofore disclosed to produce B7 or CTLA4 proteins of the present invention. Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not substantially contaminated with antibodies against other substances that might otherwise cause interference in a diagnostic assay or side effects if used in a therapeutic composition.

15 Antibodies of the present invention have a variety of potential uses that are within the scope of the present invention. For example, such antibodies can be used (a) as reagents in assays to detect B7 or CTLA4 protein, (b) as reagents in assays to modulate cellular activity through a B7 or CTLA4 protein (e.g., mimicking ligand binding to B7 or CTLA4 protein), and/or (c) as tools to screen expression libraries
20 and/or to recover desired proteins of the present invention from a mixture of proteins and other contaminants. Furthermore, antibodies of the present invention can be used to target compounds (e.g., nucleic acid molecules, drugs or proteins) to antigen presenting cells. Targeting can be accomplished by conjugating (i.e., stably joining) such antibodies to the compounds using techniques known to those skilled in the art. Suitable
25 compounds are known to those skilled in the art.

One embodiment of the present invention is a therapeutic composition that, when administered to an animal in an effective manner, is capable of regulating an immune response in an animal. Therapeutic compositions of the present invention include at least one of the following therapeutic compounds: an isolated B7 or CTLA4 protein of
30 the present invention or a mimetope thereof, an isolated B7 or CTLA4 nucleic acid molecule of the present invention, an isolated antibody that selectively binds to a B7 or

CTLA4 protein of the present invention, an inhibitor of B7 or CTLA4 function identified by its ability to bind to a B7 or CTLA4 protein of the present invention and inhibit binding of a B7 protein to CD28 and/or CTLA4, or inhibit binding of a CTLA4 protein to B7, and a mixture thereof (i.e., combination of at least two of the compounds).

- 5 As used herein, a therapeutic compound refers to a compound that, when administered to an animal in an effective manner, is able to treat, ameliorate, and/or prevent a disease. Examples of proteins, nucleic acid molecules, antibodies and inhibitors of the present invention are disclosed herein.

- 10 The present invention also includes a therapeutic composition comprising at least one B7 or CTLA4 -based compound of the present invention in combination with at least one additional protective compound. Examples of such compounds are disclosed herein.

- 15 Therapeutic compositions of the present invention can be administered to any animal susceptible to such therapy, preferably to mammals, and more preferably to dogs, cats, humans, ferrets, horses, cattle, sheep and other pets, economic food animals and/or zoo animals. Preferred animals include dogs, cats, horses and humans.

- 20 A therapeutic composition of the present invention is administered to an animal in an effective manner such that the composition is capable of regulating an immune response in that animal. Therapeutic compositions of the present invention can be administered to animals prior to onset of a disease (i.e., as a preventative vaccine) and/or can be administered to animals after onset of a disease in order to treat the disease (i.e., as a therapeutic vaccine). Preferred diseases to prevent or treat include autoimmune diseases, allergic reactions, infectious diseases, tumor development and graft rejection.

- 25 Therapeutic compositions of the present invention can be formulated in an excipient that the animal to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include
30 suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts

of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosal, o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in
5 a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

In one embodiment of the present invention, a therapeutic composition can include an adjuvant. Adjuvants are agents that are capable of enhancing the immune
10 response of an animal to a specific antigen. Suitable adjuvants include, but are not limited to, cytokines, chemokines, and compounds that induce the production of cytokines and chemokines (e.g., Flt-3 ligand, granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF),
macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF),
15 erythropoietin (EPO), interleukin 2 (IL-2), interleukin-3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), interferon gamma, interferon gamma inducing factor I (IGIF), transforming growth factor beta, RANTES (regulated upon
activation, normal T-cell expressed and presumably secreted), macrophage inflammatory
20 proteins (e.g., MIP-1 alpha and MIP-1 beta), and Leishmania elongation initiating factor (LEIF)); bacterial components (e.g., endotoxins, in particular superantigens, exotoxins and cell wall components); aluminum-based salts; calcium-based salts; silica;
polynucleotides; toxoids; serum proteins, viral coat proteins; block copolymer adjuvants (e.g., Hunter's Titermax™ adjuvant (Vaxcel™, Inc. Norcross, GA), Ribi adjuvants (Ribi
25 ImmunoChem Research, Inc., Hamilton, MT); and saponins and their derivatives (e.g., Quil A (Superfos Biosector A/S, Denmark). Protein adjuvants of the present invention can be delivered in the form of the protein themselves or of nucleic acid molecules encoding such proteins using the methods described herein.

In one embodiment of the present invention, a therapeutic composition can
30 include a carrier. Carriers include compounds that increase the half-life of a therapeutic composition in the treated animal. Suitable carriers include, but are not limited to,

polymeric controlled release vehicles, biodegradable implants, liposomes, bacteria, viruses, other cells, oils, esters, and glycols.

One embodiment of the present invention is a controlled release formulation that is capable of slowly releasing a composition of the present invention into an animal. As
5 used herein, a controlled release formulation comprises a composition of the present invention in a controlled release vehicle. Suitable controlled release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other controlled release
10 formulations of the present invention include liquids that, upon administration to an animal, form a solid or a gel *in situ*. Preferred controlled release formulations are biodegradable (i.e., bioerodible).

A preferred controlled release formulation of the present invention is capable of releasing a composition of the present invention into the blood of the treated animal at a
15 constant rate sufficient to attain therapeutic dose levels of the composition to regulate an immune response in an animal. The therapeutic composition is preferably released over a period of time ranging from about 1 to about 12 months. A controlled release formulation of the present invention is capable of effecting a treatment preferably for at least about 1 month, more preferably for at least about 3 months, even more preferably
20 for at least about 6 months, even more preferably for at least about 9 months, and even more preferably for at least about 12 months.

Therapeutic compositions of the present invention can be administered to animals prior to and/or after onset of disease. Acceptable protocols to administer therapeutic compositions in an effective manner include individual dose size, number of
25 doses, frequency of dose administration, and mode of administration. Determination of such protocols can be accomplished by those skilled in the art. A suitable single dose is a dose that is capable of regulating the immune response in an animal when administered one or more times over a suitable time period. For example, a preferred single dose of a protein, mimetope or antibody therapeutic composition is from about 1 microgram (g) to
30 about 10 milligrams (mg) of the therapeutic composition per kilogram body weight of the animal. Booster vaccinations can be administered from about 2 weeks to several

years after the original administration. Booster administrations preferably are administered when the immune response of the animal becomes insufficient to protect the animal from disease. A preferred administration schedule is one in which from about 10 g to about 1 mg of the therapeutic composition per kg body weight of the animal is administered from about one to about two times over a time period of from about 2 weeks to about 12 months. Modes of administration can include, but are not limited to, subcutaneous, intradermal, intravenous, intranasal, intraocular, oral, transdermal and intramuscular routes.

According to one embodiment, a nucleic acid molecule of the present invention can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a therapeutic protein or therapeutic RNA (e.g., antisense RNA, ribozyme, triple helix forms or RNA drug) in the animal. Nucleic acid molecules can be delivered to an animal in a variety of methods including, but not limited to, (a) administering a naked (i.e., not packaged in a viral coat or cellular membrane) nucleic acid as a genetic vaccine (e.g., as naked DNA or RNA molecules, such as is taught, for example in Wolff et al., 1990, *Science* 247, 1465-1468) or (b) administering a nucleic acid molecule packaged as a recombinant virus vaccine or as a recombinant cell vaccine (i.e., the nucleic acid molecule is delivered by a viral or cellular vehicle).

A genetic (i.e., naked nucleic acid) vaccine of the present invention includes a nucleic acid molecule of the present invention and preferably includes a recombinant molecule of the present invention that preferably is replication, or otherwise amplification, competent. A genetic vaccine of the present invention can comprise one or more nucleic acid molecules of the present invention in the form of, for example, a dicistronic recombinant molecule. Preferred genetic vaccines include at least a portion of a viral genome (i.e., a viral vector). Preferred viral vectors include those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and retroviruses, with those based on alphaviruses (such as sindbis or Semliki forest virus), species-specific herpesviruses and poxviruses being particularly preferred. Any suitable transcription control sequence can be used, including those disclosed as suitable for protein production. Particularly preferred transcription control sequences include cytomegalovirus immediate early (preferably in conjunction with Intron-A), Rous

sarcoma virus long terminal repeat, and tissue-specific transcription control sequences, as well as transcription control sequences endogenous to viral vectors if viral vectors are used. The incorporation of a "strong" polyadenylation signal is also preferred.

Genetic vaccines of the present invention can be administered in a variety of ways, with intramuscular, subcutaneous, intradermal, transdermal, intranasal and oral routes of administration being preferred. A preferred single dose of a genetic vaccine ranges from about 1 nanogram (ng) to about 600 g, depending on the route of administration and/or method of delivery, as can be determined by those skilled in the art. Suitable delivery methods include, for example, by injection, as drops, aerosolized and/or topically. Genetic vaccines of the present invention can be contained in an aqueous excipient (e.g., phosphate buffered saline) alone or in a carrier (e.g., lipid-based vehicles).

A recombinant virus vaccine of the present invention includes a recombinant molecule of the present invention that is packaged in a viral coat and that can be expressed in an animal after administration. Preferably, the recombinant molecule is packaging- or replication-deficient and/or encodes an attenuated virus. A number of recombinant viruses can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and retroviruses. Preferred recombinant virus vaccines are those based on alphaviruses (such as Sindbis virus), raccoon poxviruses, species-specific herpesviruses and species-specific poxviruses. An example of methods to produce and use alphavirus recombinant virus vaccines are disclosed in PCT Publication No. WO 94/17813, by Xiong et al., published August 18, 1994.

When administered to an animal, a recombinant virus vaccine of the present invention infects cells within the immunized animal and directs the production of a therapeutic protein or RNA nucleic acid molecule that is capable of protecting the animal from disease caused by a parasitic helminth as disclosed herein. For example, a recombinant virus vaccine comprising a B7 or CTLA4 nucleic acid molecule of the present invention is administered according to a protocol that results in the regulation of an immune response in an animal. A preferred single dose of a recombinant virus vaccine of the present invention is from about 1×10^4 to about 1×10^8 virus plaque

forming units (pfu) per kilogram body weight of the animal. Administration protocols are similar to those described herein for protein-based vaccines, with subcutaneous, intramuscular, intranasal, intraocular and oral administration routes being preferred.

A recombinant cell vaccine of the present invention includes recombinant cells
5 of the present invention that express at least one protein of the present invention.

Preferred recombinant cells for this embodiment include *Salmonella*, *E. coli*, *Listeria*, *Mycobacterium*, *S. frugiperda*, yeast, (including *Saccharomyces cerevisiae* and *Pichia pastoris*), BHK, CV-1, myoblast G8, COS (e.g., COS-7), Vero, MDCK and CRFK recombinant cells. Recombinant cell vaccines of the present invention can be
10 administered in a variety of ways but have the advantage that they can be administered orally, preferably at doses ranging from about 10^8 to about 10^{12} cells per kilogram body weight. Administration protocols are similar to those described herein for protein-based vaccines. Recombinant cell vaccines can comprise whole cells, cells stripped of cell walls or cell lysates.

15 The efficacy of a therapeutic composition of the present invention to regulate the immune response in an animal can be tested in a variety of ways including, but not limited to, detection of cellular immunity within the treated animal, determining T cell activity (helper or cytotoxic T cell activity), identify T cell repertoire, detection of IL-2 levels, detection of antibody levels, determine tumor development or challenge of the
20 treated animal with an infectious agent to determine whether the treated animal is resistant to disease. In one embodiment, therapeutic compositions can be tested in animal models such as mice. Such techniques are known to those skilled in the art.

According to the present invention, a therapeutic composition can be used to treat cancer, infectious diseases, inflammation or allergy. One of skill in the art will
25 appreciate the diseases associated with inflammation or allergy, including for example, arthritis, autoimmune diseases and atopic diseases, such as atopic dermatitis. The inventors present below novel therapeutic compositions based on the discovery of novel B7 variants. The inventors have discovered that naturally-occurring forms of B7 protein include both membrane-bound and soluble forms of B7 protein. Without being bound
30 by theory, the inventors believe that antigen presenting cells (APC's) in an animal produce both forms of B7 protein to regulate an immune response, and that the ratio of

membrane-bound to soluble B7 protein is important in such regulation. It is known that an immune response can be upregulated by activating immunoregulatory cells, e.g., T helper cells or dendritic cells, or downregulated by inactivating such immunoregulatory cells. The inventors' discovery of different forms of B7 protein suggests that such forms or derivatives thereof can be used to upregulate or downregulate an immune response. Thus, when stimulation of an immune response is desired, a therapeutic composition of the present invention can include a B7 protein that stimulates the upregulation of immunoregulatory cells and/or inhibits the downregulation of immunoregulatory cells. Alternatively, when inhibition of an immune response is desired, a therapeutic composition of the present invention can include a B7 protein that inhibits the upregulation of immunoregulatory cells and/or stimulates the downregulation of immunoregulatory cells. One of skill in the art will understand that different formulations of a therapeutic composition can be used to upregulate or downregulate an immune response as desired in a particular therapy.

One therapeutic composition of the present invention includes a B7 protein, or a nucleic acid molecule that encodes a B7 protein, that stimulates the upregulation of an immune response, i.e., activates immunoregulatory cells, referred to herein as a stimulatory activating B7 protein. A suitable stimulatory activating B7 protein includes a protein that activates T helper cell and/or T cytotoxic cell activity. A preferred stimulatory activating B7 protein comprises a membrane-bound form of a B7-1 and/or B7-2 protein of the present invention and/or a multimeric form of a naturally-occurring soluble form of B7 of the present invention. As used herein, a membrane-bound B7 protein refers to a B7 protein that is associated with a carrier, such as those disclosed herein. Preferably, a membrane-bound B7 protein is associated with the lipid bilayer of a cell in which the B7 protein has been expressed, e.g., a recombinant cell of the present invention. A preferred stimulatory activating B7 protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:27 and/or SEQ ID NO:29. A more preferred stimulatory activating B7 protein comprises the amino acid sequence SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12, SEQ ID NO:17 and/or SEQ ID NO:26. An even more preferred stimulatory activating B7 protein is encoded by a nucleic acid

molecule that comprises the nucleic acid sequence SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:25 and/or SEQ ID NO:28. A stimulatory activating protein can be delivered to an animal in the form of a protein or a nucleic acid molecule encoding the stimulatory activating protein using methods described herein.

Another therapeutic composition of the present invention includes a B7 protein, or a nucleic acid molecule that encodes a B7 protein, that stimulates the downregulation of an immune response, i.e., inactivates immunoregulatory cells. A suitable stimulatory inactivating B7 protein includes a protein that inactivates T helper cell activity, in particular mature dendritic cell activity. A preferred stimulatory inactivating B7 protein comprises a soluble form of a B7-1 and/or B7-2 protein of the present invention. A preferred stimulatory inactivating B7 protein is encoded by a nucleic acid molecule that hybridizes under stringent conditions to SEQ ID NO:13; SEQ ID NO:15, SEQ ID NO:18 SEQ ID NO:20, SEQ ID NO:33, and/or SEQ ID NO:36, or a nucleic acid molecule encoding a soluble form of a protein encoded by the complement of nucleic acid sequence SEQ ID NO:25 and/or SEQ ID NO:28. A more preferred stimulatory activating B7 protein comprises the amino acid sequence SEQ ID NO:12, SEQ ID NO:17, and/or SEQ ID NO:37, or a soluble form of the amino acid sequence SEQ ID NO:26. An even more preferred stimulatory inactivating B7 protein is encoded by a nucleic acid molecule that comprises the nucleic acid sequence SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39 and/or SEQ ID NO:40, or a nucleic acid molecule encoding a soluble form of a protein encoded by a nucleic acid sequence SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28 and/or SEQ ID NO:29.

Another therapeutic composition of the present invention includes a blocking therapeutic compound that inhibits the upregulation of downregulation of an immune response, i.e., blocks activation or inactivation of immunoregulatory cells. A blocking compound is capable of substantially interfering with the function of a B7 protein susceptible to inhibition. For example, a blocking compound is administered in an

amount and manner that inhibits an immune response to an extent that is sufficient, to treat an animal for a disease that requires downregulation of an immune response.

Suitable blocking therapeutic compounds include compounds that prevent the activation of an immunoregulatory cell through CD28 and/or CTLA4 by, for example, interfering with the binding of B7 protein to CD28 and/or CTLA4 by binding to either the B7 protein, or CD28 and/or CTLA4. Alternatively, a blocking therapeutic compound can include a compound that binds to CD28 and/or CTLA4 but does not result in activation of a T cell upon binding. Preferably, a blocking compound is derived from a B7 or CTLA4 protein of the present invention. Examples of blocking compounds include an antibody of the present invention, that is administered to an animal in an effective manner (i.e., is administered in an amount so as to be present in the animal at a titer that is sufficient, upon interaction of that antibody with a native B7 or CTLA4 protein, to decrease B7 or CTLA4 activity in an animal, at least temporarily). Oligonucleotide nucleic acid molecules of the present invention can also be administered in an effective manner, thereby reducing expression of B7 or CTLA4 proteins in order to interfere with B7 or CTLA4 activity targeted in accordance with the present invention. Peptides of B7 or CTLA4 proteins of the present invention can also be administered in an effective manner, thereby reducing binding of B7 proteins to CD28 and/or CTLA4, or reducing binding of CTLA4 to B7, in order to interfere with B7 or CTLA4 activity targeted in accordance with the present invention. A blocking compound of B7 or CTLA4 function can be identified using B7 or CTLA4 proteins of the present invention. One embodiment of the present invention is a method to identify a compound capable of inhibiting B7 or CTLA4 function. Such a method includes the steps of: (a) contacting (e.g., combining, mixing) an isolated B7 or CTLA4 protein of the present invention, with a putative inhibitory compound under conditions in which, in the absence of the compound, the B7 protein binds to CD28 or CTLA4, or the CTLA4 protein binds to B7, and (b) determining if the putative inhibitory compound inhibits the binding of B7 protein to CD28 or CTLA4, or the binding of CTLA4 protein to B7. Putative inhibitory compounds to screen include small organic molecules, antibodies (including mimetopes thereof), and ligand analogs. Such compounds are also screened to identify those that are substantially not toxic in host animals.

Inhibitors of B7 or CTLA4 function identified by such a method can be tested for their ability to block T cell activation *in vivo*. Preferred B7 or CTLA4 proteins to inhibit are those produced by dogs, cats, horses or humans, even more preferred B7 or CTLA4 proteins to inhibit are those produced by domestic dogs or cats. A particularly preferred inhibitor of the present invention is capable of regulating an immune response in an animal. It is also within the scope of the present invention to use inhibitors of the present invention to target diseases involving undesired helper T cell activity in animals. Compositions comprising inhibitors of B7 or CTLA4 function can be administered to animals in an effective manner to regulate the immune response in the animals, and preferably to prevent autoimmune disease, allergy or prevent graft rejection in animals, or to treat animals with such diseases. Effective amounts and dosing regimens can be determined using techniques known to those skilled in the art.

According to the present invention, different therapeutic compositions of the present invention can be used to administer to patients having a disease. In one embodiment, a therapeutic composition comprising a nucleic acid molecule that encodes a stimulatory activating B7 protein and/or stimulatory inactivating B7 protein is administered to an animal having cancer. Suitable nucleic acid molecules, and methods to deliver and express such molecules in an animal, are disclosed herein. Preferably, a nucleic acid molecule encoding a stimulatory inactivating B7 protein is delivered in amounts sufficient to produce low doses of stimulatory inactivating B7 protein. Preferably, a nucleic acid molecule encoding a stimulatory activating B7 protein is delivered in amounts sufficient to produce low doses of stimulatory activating B7 protein.

In another embodiment, a therapeutic composition comprising a nucleic acid molecule encoding a stimulatory activating B7 protein is administered to an animal susceptible to or having an infectious disease. Suitable nucleic acid molecules, and methods to deliver and express such molecules in an animal, are disclosed herein. Preferably, a nucleic acid molecule encoding a stimulatory activating B7 protein is delivered in amounts sufficient to produce low doses of stimulatory activating B7 protein. An infectious disease therapy preferably includes co-administration of an antigen derived from the pathogen causing an infectious disease. Suitable antigens can

be selected by one of skill in the art depending on the pathogen, and can include parasites, viruses, bacteria and fungi. Preferred parasite antigens include heartworm; *Yersinia*, *Pasteurella*, *Francisella*, fleas; ticks, including both hard ticks of the family Ixodidae (e.g., *Ixodes* and *Amblyomma*) and soft ticks of the family Argasidae (e.g.,
5 *Ornithodoros*, such as *O. parkeri* and *O. turicata*); flies, such as midges (e.g., *Culicoides*), mosquitos, sand flies, black flies, horse flies, horn flies, deer flies, tsetse flies, stable flies, myiasis-causing flies and biting gnats; ants; spiders, lice; mites; and true bugs, such as bed bugs and kissing bugs, including those carrying Chagas disease. Preferred viruses include FeLV and FIV. In addition, an infectious disease therapy can
10 include co-administration of a compound capable of shifting an immune response to a TH1 response. An example of such a compound is IL-12.

In another embodiment, a therapeutic composition comprising a high concentration of stimulatory activating and stimulatory inactivating B7 protein is administered to an animal having inflammation and/or autoimmune disease. Suitable
15 proteins and methods to deliver and express such proteins in an animal are disclosed herein. Preferably, a stimulatory activating and stimulatory inactivating B7 protein is delivered to a localized area in an animal that is afflicted with inflammation or autoimmune disease. Localized areas can include, for example, joints or organs. As used herein, the term organ includes skin and lung, and other tissues understood to be
20 organs by those of skill in the art.

In another embodiment, a therapeutic composition comprising a protein, and/or a nucleic acid molecule encoding a protein, including a stimulatory activating and stimulatory inactivating B7 protein, is administered to an animal having an allergy. Suitable proteins and nucleic acid molecules, and methods to deliver and express such
25 proteins and nucleic acid molecules in an animal, are disclosed herein. Doses of an allergy therapy can vary depending upon the allergy being treated and the location of an allergic response. For example, one dose may be suitable for treatment of an allergy requiring topical administration compared with a dose suitable for treatment of an allergy requiring inhaled administration. One of skill in the art can select appropriate
30 doses based on the allergy and mode of administration being used.

It is also within the scope of the present invention to use isolated proteins, mimetopes, nucleic acid molecules and antibodies of the present invention as diagnostic reagents. Methods to use such diagnostic reagents are well known to those skilled in the art.

- 5 The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

- It is to be noted that the examples include a number of molecular biology, microbiology, immunology and biochemistry techniques considered to be familiar to those skilled in the art. Disclosure of such techniques can be found, for example, in 10 Sambrook et al., *ibid.* and Ausubel, et al., 1993, *Current Protocols in Molecular Biology*, Greene/Wiley Interscience, New York, NY, and related references. It should also be noted that since nucleic acid sequencing technology, and in particular the sequencing of PCR products, is not entirely error-free, that the nucleic acid and deduced 15 protein sequences presented herein represent apparent nucleic acid sequences of the nucleic acid molecules encoding B7 or CTLA4 proteins of the present invention.

Example 1

This example describes the isolation and DNA sequencing of two forms of *Canis familiaris* B7-1 nucleic acid molecules.

- 20 A *C. familiaris* mitogen activated PBMC cDNA library was constructed in the Uni-ZAP® XR vector, available from Stratagene Cloning Systems, La Jolla, CA, using Stratagene's ZAP-cDNA® Synthesis Kit and the manufacturer's protocol. The mRNA was isolated from *C. familiaris* peripheral blood mononuclear cells 4 hours after they were activated by a polyclonal activating agent in culture. A first polymerase chain 25 reaction (PCR) product corresponding to a 3' portion of a canine B7-1 nucleic acid molecule was produced as follows. A pair of primers was used to amplify DNA from the cDNA library. A 3' T7 primer from the Uni-ZAP® XR vector, available from Stratagene, was used in combination with a degenerate primer, the design of which was based on conserved regions of B7-1 cDNA sequences from other species in the public 30 databases corresponding to the positions shown below:

Database	Accession number	Nucleotides	Animal
gb	U57755	412-429	cat
gb	M27533	729-746	human
gb	U10925	708-725	rat
dbj	D49843	430-447	rabbit
gb	U19840	412-429	rhesus monkey

5 The degenerate primer is a 5' primer having the nucleic acid sequence 5' GTC ARA GCT GAC TTC CCT 3', where R can be either A or G, designated herein as SEQ ID NO:21. This degenerate primer for B7-1 was one of several degenerate primers tested
10 and was the only primer that was found to be useful for the production of a canine B7-1 nucleic acid molecule. Initial attempts using a combination of several pairs of degenerate B7-1 primers to directly amplify fragments of canine B7-1 nucleic acid molecules from the cDNA library or from mRNA by RT/PCR were unsuccessful. The PCR product was cloned and sequenced using an Applied Biosystems, Inc. automated
15 DNA sequencer using standard methods.

A second PCR product corresponding to a 5' portion of a canine B7-1 nucleic acid molecule was produced as follows. A pair of primers was used to amplify DNA from the cDNA library. An antisense primer was designed using sequence derived from the first PCR product, the primer having the nucleic acid sequence 5' GTA GAA ACT
20 CCT CAG AAC AAT G 3' (designated herein as SEQ ID NO:22), was used in combination with the 5' vector primer M13 Rev, available from Life Technologies, Gaithersburg, MD. This second PCR fragment was cloned and sequenced using standard methods.

To identify full-length B7-1 clones, the second PCR product was used to
25 generate an about 545 base pair DNA fragment. The fragment was then labeled with ³²P and used as a probe to screen the canine PBMC cDNA library. Hybridization was done at about 68°C in 6XSSC, 5X Denhardt's solution, 0.5% SDS, 100 µg/ml of salmon sperm DNA and yeast tRNA. The blot was washed two times, for about 30 minutes per wash, at 55°C in 1XSSC, 0.1%SDS. Positive clones were isolated and the cDNA inserts
30 were sequenced for both strands using vector flanking primers and gene-specific internal primers. Sequence analysis was performed with DNAsis™ using the alignment settings

of: gap penalty set at 5; number of top diagonals set at 5; fixed gap penalty set at 10; k-tuple set at 2; window size set at 5 and floating gap penalty set at 10.

A. A first clone (Clone 14) was isolated, referred to herein as nCaB7-1₂₈₃₀, contained in a recombinant molecule referred to herein as λ -nCaB7-1₂₈₃₀, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:1. The complement of SEQ ID NO:1 is represented herein by SEQ ID NO:3. Translation of SEQ ID NO:1 suggests that nucleic acid molecule nCaB7-1₂₈₃₀ encodes a full-length B7-1 protein of about 304 amino acids, denoted herein as PCaB7-1₃₀₄, the amino acid sequence of which is presented in SEQ ID NO:2, assuming an open reading frame having an initiation codon spanning from nucleotide 337 through nucleotide 339 of SEQ ID NO:1 and a stop codon spanning from nucleotide 1249 through nucleotide 1251 of SEQ ID NO:1. The coding region encoding PCaB7-1₃₀₄ is presented herein as nCaB7-1₉₁₂, which has the nucleotide sequence SEQ ID NO:4 (the coding strand) and SEQ ID NO:5 (the complementary strand). A putative signal sequence extends from amino acid residue 1 through residue 34 of SEQ ID NO:2. The proposed mature protein (i.e., canine B7-1 protein from which the signal sequence has been cleaved), denoted herein as PCaB7-1₂₇₀, contains about 270 amino acids, extending from residue 35 through residue 304 of SEQ ID NO:2. The nucleic acid molecule encoding PCaB7-1₂₇₀ is denoted herein as nCaB7-1₈₁₀, extending from nucleotide 102 through nucleotide 912 of SEQ ID NO:4.

Comparison of nucleic acid sequence SEQ ID NO:1 with nucleic acid sequences reported in GenBank indicates that SEQ ID NO:1 showed the most homology, i.e., about 79.7% identity, between SEQ ID NO:1 and a feline B7-1 gene. Comparison of amino acid sequence SEQ ID NO:2 with amino acid sequences reported in GenBank indicates that SEQ ID NO:2 showed the most homology, i.e., about 79.1% identity, between SEQ ID NO:2 and a feline B7-1 protein.

B. A second clone (Clone 22) was isolated, referred to herein as nCaB7-1s₁₀₂₄, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:11. The complement of SEQ ID NO:11 is referred to herein as SEQ ID NO:13. Translation of SEQ ID NO:11 suggests that nucleic acid molecule nCaB7-1s₁₀₂₄ encodes a variant form of B7-1 protein of about 235 amino acids, denoted

herein as PCaB7-1s₂₃₅, the amino acid sequence of which is presented in SEQ ID NO:12, assuming an open reading frame having an initiation codon spanning from nucleotide 79 through nucleotide 81 of SEQ ID NO:11 and a stop codon spanning from nucleotide 784 through nucleotide 786 of SEQ ID NO:11. The coding region encoding PCaB7-1s₂₃₅,
 5 not including the termination codon, is presented herein as nCaB7-1s₇₀₅, which has the nucleotide sequence SEQ ID NO:14 (the coding strand) and SEQ ID NO:15 (the complementary strand). A putative signal sequence extends from amino acid residue 1 through residue 34 of SEQ ID NO:12. The proposed mature protein (i.e., canine soluble B7-1 protein from which the signal sequence has been cleaved), denoted herein as
 10 PCaB7-1s₂₀₁, contains about 201 amino acids, extending from residue 35 through residue 235 of SEQ ID NO:12. The nucleic acid molecule encoding PCaB7-1s₂₀₁ is denoted herein as nCaB7-1s₆₀₃, extending from nucleotide 103 through nucleotide 705 of SEQ ID NO:14.

The open reading frame (ORF) of nCaB7-1s₁₀₂₄ encodes a polypeptide of 235
 15 amino acid residues, which is 69 residues shorter than the protein encoded by the nucleic acid molecule nCaB7-1₂₈₃₀, which is 304 amino acid residues in length. Nucleotides 1 through 699 of nCaB7-1₂₈₃₀ are shared by nucleic acid molecules nCaB7-1s₁₀₂₄ and nCaB7-1₂₈₃₀, which corresponds to the extracellular domain of B7-1. Nucleotides 700 through 912 of nCaB7-1₂₈₃₀ are deleted in the nucleic acid molecule nCaB7-1s₁₀₂₄. Thus,
 20 nucleic acid molecule nCaB7-1s₁₀₂₄ lacks portions of the transmembrane and cytoplasmic domains of B7-1 present in nCaB7-1₂₈₃₀. The sequence of nucleic acid molecules nCaB7-1s₁₀₂₄ and nCaB7-1₂₈₃₀ indicates that nCaB7-1₂₈₃₀ encodes a soluble B7-1 protein and that nCaB7-1₂₈₃₀ encodes a membrane bound form of B7-1. The 3' untranslated sequence of nCaB7-1₂₈₃₀, however, differs from nCaB7-1s₁₀₂₄. This
 25 suggests that the different forms of canine B7-1 are likely generated by alternate RNA splicing.

Example 2

This example describes the isolation and DNA sequencing of two forms of *Canis familiaris* B7-2 nucleic acid molecules.

30 The canine PBMC cDNA library constructed as in Example 1 was used to identify and isolate B7-2 nucleic acid molecules using the following method. As a first step, a 322-

bp PCR fragment encoding a portion of canine B7-2 was amplified using two degenerate primers. The primers were designed based on conserved regions of the B7-2 gene of mouse, rat and human.

Primer	Database	Accession number	Nucleotides	Animal
F1	gb	L25606	258-276	mouse
F1	gb	U31330	21-39	rat
F1	gb	L25259	266-284	human
B1	gb	L25606	576-554	mouse
B1	gb	U31330	339-317	rat
B1	gb	L25259	584-562	human

A sense degenerate primer, referred to as F1, having the nucleic acid sequence 5' GTA GTA TTT TGG CAG GAC C 3' (designated SEQ ID NO:23), was used in combination with an antisense degenerate primer, referred to as B1, having the nucleic acid sequence 5' TAG AYG SGC AGG TCA AAT TTA TG 3' (designated SEQ ID NO:24), where Y can be either C or T, and S can be either C or G. Initial attempts using numerous degenerate B7-2 primers to directly amplify fragments of canine B7-2 nucleic acid molecules from the cDNA library were unsuccessful. The resulting 322-bp PCR product was cloned and sequenced using an Applied Biosystems, Inc. automated DNA sequencer using standard methods.

To identify full-length B7-2 clones, the 322-bp PCR product was labeled with 32P and used as a probe to screen the canine PBMC cDNA library. Hybridization was performed as described in Example 1. Positive clones were isolated and the cDNA inserts were sequenced for both strands using vector flanking primers and gene-specific internal primers. Sequence analysis was performed with DNAsis™ using the settings described in Example 1.

A. A clone (Clone 10) was isolated, referred to herein as nCaB7-2₁₈₉₇, contained in a recombinant molecule referred to herein as λ -nCaB7-2₁₈₉₇, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:6. The complement of SEQ ID NO:6 is represented herein by SEQ ID NO:8.

Translation of SEQ ID NO:6 suggests that nucleic acid molecule nCaB7-2₁₈₉₇ encodes a full-length B7-2 protein of about 329 amino acids, denoted herein as PCaB7-2₃₂₉, the

amino acid sequence of which is presented in SEQ ID NO:7, assuming an open reading frame having an initiation codon spanning from nucleotide 6 through nucleotide 8 of SEQ ID NO:6 and a stop codon spanning from nucleotide 993 through nucleotide 995 of SEQ ID NO:6. The coding region encoding PCaB7-2₃₂₉, not including the termination

5 codon, is presented herein as nCaB7-2₉₈₇, which has the nucleotide sequence SEQ ID NO:9 (the coding strand) and SEQ ID NO:10 (the complementary strand). A putative signal sequence extends from amino acid residue 1 through residue 22 of SEQ ID NO:6. The proposed mature protein (i.e., canine B7-2 protein from which the signal sequence has been cleaved), denoted herein as PCaB7-2₃₀₇, contains about 307 amino acids,

10 extending from residue 23 through residue 329 of SEQ ID NO:7. The nucleic acid molecule encoding PCaB7-2₃₀₇ is denoted herein as nCaB7-2₉₂₁, extending from nucleotide 66 through nucleotide 987 of SEQ ID NO:9.

Comparison of nucleic acid sequence SEQ ID NO:6 with nucleic acid sequences reported in GenBank indicates that SEQ ID NO:6 showed the most homology, i.e., about

15 77.2% identity, between SEQ ID NO:6 and a swine B7-2 gene. Comparison of amino acid sequence SEQ ID NO:7 with amino acid sequences reported in GenBank indicates that SEQ ID NO:7 showed the most homology, i.e., about 56.3% identity, between SEQ ID NO:7 and a swine B7-2 protein.

B. Another clone (Clone 1) was isolated, referred to herein as nCaB7-2s₁₇₉₅,

20 the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:16. The complement of SEQ ID NO:16 is represented herein by SEQ ID NO:18. Translation of SEQ ID NO:16 suggests that nucleic acid molecule nCaB7-2s₁₇₉₅ encodes a variant form of B7-2 protein of about 280 amino acids, denoted herein as PCaB7-2s₂₈₀, the amino acid sequence of which is presented in SEQ ID NO:17,

25 assuming an open reading frame having an initiation codon spanning from nucleotide 7 through nucleotide 9 of SEQ ID NO:16 and a stop codon spanning from nucleotide 847 through nucleotide 849 of SEQ ID NO:16. The coding region encoding PCaB7-2s₂₈₀, not including the termination codon, is presented herein as nCaB7-2s₈₄₀, which has the nucleotide sequence SEQ ID NO:19 (the coding strand) and SEQ ID NO:20 (the

30 complementary strand). SEQ ID NO:16 is predicted to encode a B7-2 protein lacking at least a portion of the transmembrane domain. A putative signal sequence extends from

amino acid residue 1 through residue 22 of SEQ ID NO:17. The proposed mature protein (i.e., canine soluble B7-2 protein from which the signal sequence has been cleaved), denoted herein as PCaB7-2s₂₅₈, contains about 258 amino acids, extending from residue 23 through residue 280 of SEQ ID NO:17. The nucleic acid molecule
 5 encoding PCaB7-2s₂₅₈ is denoted herein as nCaB7-2s₇₇₄, extending from nucleotide 66 through nucleotide 840 of SEQ ID NO:19.

C. The ORF of nCaB7-2s₁₇₉₅ encodes a polypeptide of 280 amino acid residues, which is 49 residues shorter than the protein encoded by the nucleic acid molecule nCaB7-2₁₈₉₇, which is 329 amino acid residues in length. Nucleotides 1
 10 through 702 of nCaB7-2₁₈₉₇ are shared by nucleic acid molecules nCaB7-2s₁₇₉₅ and nCaB7-2₁₈₉₇, which corresponds to the extracellular domain of B7-2. Nucleotides 703 through 852 of nCaB7-2₁₈₉₇ are deleted in the nucleic acid molecule nCaB7-2s₁₇₉₅, which corresponds to a portion of the transmembrane domain of B7-2. Nucleotides 853 through 907 of nCaB7-2₁₈₉₇ are identical to nucleotides 703 through 840 of nucleic acid
 15 molecule nCaB7-2s₁₇₉₅, which corresponds to the intracellular domain of B7-2. Thus, nucleic acid molecule nCaB7-2t₁₇₉₅ lacks a portion of the transmembrane domain of B7-2 present nCaB7-2₁₈₉₇. The sequence of nucleic acid molecules nCaB7-2s₁₇₉₅ and nCaB7-2₁₈₉₇ indicates that nCaB7-2s₁₇₉₅ encodes a soluble B7-2 protein and that nCaB7-2₁₈₉₇ encodes a membrane bound form of B7-2. The different forms of canine B7-2 are
 20 encoded by contiguous sequence, differing by the location of their respective termination codons. The different locations of termination codons may be due to alternative RNA splicing, due to the existence of two different genes or due to DNA recombination.

A search of the databases revealed that predicted amino acid sequences of the canine B7-1 and B7-2 proteins, similar to those of mouse B7 proteins, shared homology
 25 with members of the immunoglobulin (Ig) superfamily due to the presence of two contiguous Ig-like domains in the extracellular region. The first canine B7 Ig domain is consistent with the structure of an Ig V domain. The second canine B7 Ig domain is consistent with the structure of an Ig C domain. For the full-length canine B7-1, the IgV-like domain extends from residue 34 to residue 139 SEQ ID NO:2, and the IgC-like
 30 domain extends from residue 140 to residue 233 of SEQ ID NO:2. For the secreted canine B7-1, the IgV-like domain extends from residue 34 to residue 139 SEQ ID

NO:12, and the IgC-like domain extends from residue 140 to residue 235 of SEQ ID NO:12.

Example 3

This example describes the production of recombinant molecules encoding different forms of canine B7-1 and B7-2 proteins, and the expression of these recombinant molecules in mammalian cells.

A. A recombinant expression plasmid, denoted herein as pCMV-nCaB7-2₁₈₉₇, capable of expressing a full length form of B7-2, was produced as follows. Recombinant molecule λ -nCaB7-2₁₈₉₇ was digested with the restriction endonucleases *Bam*H I and *Xho* I. The 1897-bp insert encoding full-length B7-2 was gel purified and ligated into the HCMV immediate-early transcription control region of the pCMV-Int A plasmid vector that had been digested with *Bam*H I and *Xho* I and gel purified, to produce the recombinant molecule pCMV-nCaB7-2₁₈₉₇. The insert size and identity were confirmed by restriction digestion, PCR, and sequencing. The pCMV-Int A plasmid vector was produced as follows. Vector pRc/RSV, available from Invitrogen Corp., San Diego, CA, was cleaved by restriction enzyme with *Pvu*II, and the 2963-base pair *Pvu*II fragment was gel purified. The fragment was self-ligated to form the vector pRc/RSV(*Pvu*), which contains a Rous Sarcoma Virus (RSV) long terminal repeat, a multiple cloning site, a bovine growth hormone polyadenylation sequence, a bacterial origin of replication, and an ampicillin resistance gene. Vector pRc/RSV(*Pvu*) was restriction enzyme digested using *Hind*III and *Nru*I. A *Hind*III/*Ssp*I fragment containing the HCMV intermediate early promoter and first intron (i.e., intron A) was ligated into the digested pRc/RSV(*Pvu*) vector to produce the vector pCMV-Int A.

B. A second recombinant expression clone, denoted herein as pCMV-nCaB7-1₁₃₈₅, capable of expressing a full length form of B7-1, was produced as follows. Nucleic acid molecule λ -nCaB7-1₂₈₃₀ was digested with the restriction endonuclease *Pst*I to produce a 1385-bp *Pst*I fragment. The 1385-bp fragment encoding full-length B7-1 was gel purified and ligated into the plasmid vector pCMV-Int A that had been digested with *Pst*I and gel purified, to produce the recombinant molecule pCMV-nCaB7-1₁₃₈₅. Insert size and identity were confirmed by restriction digestion, PCR, and sequencing.

C. Stable expression of recombinant canine B7-1 and B7-2 in mammalian cells carrying the recombinant plasmids, pCMV-nCaB7-2₁₈₉₇ or pCMV-nCaB7-1₁₃₈₅ was demonstrated by introducing these plasmids into Chinese Hamster Ovary cells (CHO, available from ATCC, as follows. Briefly, six-well polystyrene tissue culture plates were seeded with approximately 5×10^5 /well in 2 ml of MEM, available from Life Technologies, supplemented with 100 mM L-glutamine, gentamicin, and 10% FBS (TCM). Cells were grown to about 80% confluence (about 18 hrs). The recombinant molecules to be transfected were purified using the 5' Prime to 3' Prime Kit, available from 5' to 3', Inc., Boulder, CO, as per the manufacturer's instructions. The recombinant plasmids were linearized using the restriction enzyme *PvuI*. The plasmid pcDNA3, available from Invitrogen, which contains the neomycin resistance gene, was linearized with the restriction enzyme *EcoRI*. Approximately 2 μ g of pCMV-nCaB7-2₁₈₉₇ or pCMV-nCaB7-1₁₃₈₅, were mixed separately with about 2 ng of pcDNA3 in about 100 μ l OptiMEM medium, available from Life Technologies. About 10 μ l Lipofectamine, available from Life Technologies, was mixed with about 100 μ l OptiMEM. The plasmid mixture was then added to the Lipofectamine mixture and incubated at room temperature for about 45 min. After incubation, about 800 μ l of OptiMEM was added, and the entire mixture was overlaid onto the CHO cells that had been rinsed with OptiMEM. Cells were incubated for about 5 hours at 37° C, 5% CO₂, 95% relative humidity. Approximately 1 ml of TCM with 20% FBS was added, and the cells were incubated overnight. The media was changed after about 24 hr. About 72 hr post transfection, the cells were split 1:4 and put into selection TCM containing about 400 μ g/ml geneticin (G418), available from Life Technologies. The media was changed every 3-5 days. After several weeks, G418-resistant colonies were trypsinized using cloning cylinders, and the cells were plated into 24 well plates. The resulting recombinant cells are referred to herein as CHO-pCMV-nCaB7-2₁₈₉₇ and CHO-pCMV-nCaB7-1₁₃₈₅, respectively. The recombinant cells were then expanded for testing.

Example 4

This example describes the mitogen costimulatory activity of B7-2 protein expressed in CHO-pCMV-nCaB7-2₁₈₉₇ or B7-1 protein expressed in CHO-pCMV-nCaB7-1₁₃₈₅ cells.

The stable transfectants produced in Example 3 were tested in a mitogen costimulatory assay for expression of full length B7 molecules as follows. Briefly, CHO-pCMV-nCaB7-2₁₈₉₇ or CHO-pCMV-nCaB7-1₁₃₈₅ cells were trypsinized and plated into 96 well flat bottom plates at about 5×10^4 cells/well. After about 18-24 hrs, the

5 CHO-pCMV-nCaB7-2₁₈₉₇ or CHO-pCMV-nCaB7-1₁₃₈₅ cells were fixed with about 1% paraformaldehyde in phosphate buffered saline (PBS) for about 15 mins at room temperature and then washed extensively with PBS. About 5×10^3 to about 5×10^4 resting canine T cells were added to the wells containing the CHO-pCMV-nCaB7-2₁₈₉₇ or CHO-pCMV-nCaB7-1₁₃₈₅ cells in the absence or presence of approximately 0.1 to

10 1 $\mu\text{g/ml}$ Concanavalin A (Con A; available from Sigma, St. Louis, MO). The cultures were incubated at about 37° C, 5% CO₂, 95% relative humidity for about 5 to 7 days. About 1 μCi tritiated thymidine was added to each well for the last 18 to 24 hrs of the incubation. The cells were harvested onto filtermats which were then counted. The stimulation indices using 1 or 0.1 $\mu\text{g/ml}$ Con A are shown below in Tables 1 and 2.

15 Table 1. Costimulation of resting canine T cells by Recombinant B7-2 Protein

Con A	CHO	B7-2 Transfectant	B7-2 Transfectant
1.0 $\mu\text{g/ml}$	3	14	16
0.1 $\mu\text{g/ml}$	2	19	16
None	2	1	1

20 Table 2. Costimulation of resting canine T cells by Recombinant B7-1 Molecules

Con A	CHO	B7-1 Transfectant	B7-1 Transfectant
1.0 $\mu\text{g/ml}$	3	13	50
0.1 $\mu\text{g/ml}$	2	12	34
None	1	1	1

25 The results indicated that the B7-2 or B7-1 recombinant proteins expressed by recombinant cells CHO-pCMV-nCaB7-2₁₈₉₇ or CHO-pCMV-nCaB7-1₁₃₈₅, respectively, are capable of stimulating resting T cells.

Example 5

This example describes the isolation and DNA sequencing of *Felis* B7-2 nucleic acid molecules.

- A *Felis* mitogen activated PBMC cDNA library was constructed in the Uni-
- 5 ZAP® XR vector using the methods described above in Example 1. As a first step in the isolation of full-length feline B7-2 cDNA, a 322-bp PCR fragment encoding a portion of feline B7-2 was amplified from the cDNA library using the two degenerate primers described above in Example 2. To identify full-length B7-2 clones, the 322-bp PCR product was labeled with ³²P and used as a probe to screen the feline PBMC cDNA
 - 10 library using the hybridization conditions described above in Example 1. Positive clones were isolated and the cDNA inserts were sequenced for both strands using vector flanking primers and gene-specific internal primers. Sequence analysis was performed with DNAsis™ using the settings described above in Example 1.

- A clone (Clone 4-6) was isolated, referred to herein as nFeB7-2₂₈₃₀, contained in
- 15 a recombinant molecule referred to herein as λ-nFeB7-2₂₈₃₀, the coding strand of which was shown to have a nucleic acid sequence denoted herein as SEQ ID NO:25. The complement of SEQ ID NO:25 is represented herein by SEQ ID NO:27. Translation of SEQ ID NO:25 suggests that nucleic acid molecule nFeB7-2₂₈₃₀ encodes a full-length B7-2 protein of about 332 amino acids, denoted herein as PFeB7-2₃₃₂, the amino acid
 - 20 sequence of which is presented in SEQ ID NO:26, assuming an open reading frame having an initiation codon spanning from nucleotide 179 through nucleotide 181 of SEQ ID NO:25 and a stop codon spanning from nucleotide 1175 through nucleotide 1177 of SEQ ID NO:25. The coding region encoding PFeB7-2₃₃₂, not including the termination codon, is presented herein as nFeB7-2₉₉₆, which has the nucleotide sequence SEQ ID
 - 25 NO:28 (the coding strand) and SEQ ID NO:29 (the complementary strand). A putative signal sequence extends from amino acid residue 1 through residue 23 of SEQ ID NO:26. The proposed mature protein (i.e., feline B7-2 protein from which the signal sequence has been cleaved), denoted herein as PFeB7-2₃₀₉, contains about 309 amino acids, extending from residue 24 through residue 329 of SEQ ID NO:26. The nucleic
 - 30 acid molecule encoding PFeB7-2₃₀₉ is denoted herein as nFeB7-2₉₁₈, extending from nucleotide 69 through nucleotide 996 of SEQ ID NO:28.

Comparison of nucleic acid sequence SEQ ID NO:25 with nucleic acid sequences reported in GenBank indicates that SEQ ID NO:25 showed the most homology, i.e., about 76.9% identity, between SEQ ID NO:25 and a swine B7-2 gene.

Comparison of amino acid sequence SEQ ID NO:26 with amino acid sequences reported in GenBank indicates that SEQ ID NO:26 showed the most homology, i.e., about 57.5% identity, between SEQ ID NO:26 and a swine B7-2 protein.

Example 6

This example describes the identification of transcripts encoding a soluble form of feline B7-2.

10 In order to identify different forms of feline B7-2, first strand cDNA was synthesized from *felis* lymph node and spleen cells using methods described above in Example 1. The cDNA was used as a template for PCR with feline B7-2 specific sense primers 5'ATA CAA GGT TAC CCA GAA CC 3' (designated herein as SEQ ID NO:51), corresponding to nucleotides 662-681 of SEQ ID NO: 25; and antisense primer
15 5' TGT GTA GTA CTT TTG TCG CC 3' (designated herein as SEQ ID NO:52), corresponding to the reverse complement of nucleotides 1151-1170 of SEQ ID NO:25. The PCR reaction resulted in the amplification of two nucleic acid molecules, having sizes of about 509 base-pairs and about 359 base-pairs, respectively. The two PCR products were cloned and subjected to nucleotide sequencing using standard methods.

20 The larger fragment, denoted herein as nFeB7-2₅₀₉, has a coding strand corresponding to nucleotides 662-1170 of SEQ ID NO:25, represented herein as SEQ ID NO:30. The complement of SEQ ID NO:30 is represented herein as SEQ ID NO:32. Nucleic acid molecule nFeB7-2₅₀₉ encodes a B7-2 protein of about 169 amino acids, denoted herein as PFeB7-2₁₆₉, the amino acid sequence of which is presented in SEQ ID
25 NO:31, assuming an open reading frame spanning from nucleotide 1 through nucleotide 507 of SEQ ID NO:30. SEQ ID NO:31 corresponds to amino acids 162-330 of SEQ ID NO:26.

The smaller fragment, denoted herein as nFeB7-2s₃₅₉, has a coding strand represented herein as SEQ ID NO:33. The complement of SEQ ID NO:33 is represented
30 herein as SEQ ID NO:35. SEQ ID NO:33 is identical to SEQ ID NO:30, except for a deletion of 150 nucleotides extending from nucleotide 226 to nucleotide 375 of SEQ ID

NO:30, and from nucleotide 887 to nucleotide 1036 of SEQ ID NO:25. Nucleic acid molecule nFeB7-2s₃₅₉ encodes a B7-2 protein of about 119 amino acids, denoted herein as PFeB7-2s₁₁₉, the amino acid sequence of which is presented in SEQ ID NO:34, assuming an open reading frame spanning from nucleotide 1 through nucleotide 357 of

5 SEQ ID NO:30.

SEQ ID NO:30 and SEQ ID NO:33 were aligned with the canine B7-2 nucleic acid molecules isolated as described in Example 2. The nucleotides deleted in SEQ ID NO:33 relative to SEQ ID NO:30 corresponded to the nucleotides deleted in nucleic acid molecule nCaB7-2s₁₇₉₅ (SEQ ID NO:16), which encodes a soluble canine B7-2, relative

10 to nCaB7-2₁₈₉₇ (SEQ ID NO:6), that encodes a membrane bound form of B7-2. The length of the deleted fragment in nFeB7-2s₃₅₉ was 150 nucleotides and that in nCaB7-2s₁₇₉₅ was 147 nucleotides. This suggests that the transcript encoding soluble feline B7-2 is generated by the same mechanism that was described for canine B7-2 in Example 2.B., i.e., by deletion of the transmembrane domain exon.

15 Example 7

This example describes the identification and cloning of a cDNA molecule encoding a truncated form of feline B7-1.

In order to identify different forms of feline B7-1, the *Felis* PBMC cDNA library, constructed as described in Example 5, was used as a template for PCR with feline B7-

20 1-specific sense primer 5' GGG AAT TCG CCA CCA TGG GTC ACG CAG CAA AGT G 3', designated herein as SEQ ID NO:53, with nucleotides 15-34 corresponding to nucleotides 1-20 of GenBank accession number U57755; and as the antisense primer either: feline B7-1-specific antisense primer 5' CCC TCG AGC TAT GTA GAC AGG TGA GAT C 3', designated herein as SEQ ID NO:54, with nucleotides 9-28

25 corresponding to the reverse complement of nucleotides 860-879 of GenBank accession number U57755; or the λ -Zap vector primer T7 5' GTA ATA CGA CTC ACT ATA GGG C 3', designated herein as SEQ ID NO:55, available from Stratagene. To facilitate translation in eukaryotic cells and cloning into pCMV-IntA as described in Example 8, an *Eco* RI site and Kozak translation start sequence (shown in bold) were added to the

30 sense primer SEQ ID NO:53, and an *Xho* I site (shown in bold) was added to the antisense primer SEQ ID NO:54. The fragment amplified using the two feline B7-1-

specific primers was 879 nucleotides in length, corresponding to the predicted coding region of full-length feline B7-1. When the λ -Zap vector primer T7 was used, a predominant fragment of 594 base-pairs was amplified. The 594-base-pair fragment was cloned and subjected to nucleotide sequencing using standard methods.

5 The cloned 594-base-pair nucleic acid molecule, referred to herein as nFeB7-1s₅₉₄, has a coding strand having the nucleic acid sequence denoted herein as SEQ ID NO:36. The reverse complement of SEQ ID NO:36 is represented herein by SEQ ID NO:38. Translation of SEQ ID NO:36 suggests that nucleic acid molecule nFeB7-1s₅₉₄ encodes a truncated B7-1 protein of about 173 amino acids, denoted herein
10 as PFeB7-1s₁₇₃, the amino acid sequence of which is presented in SEQ ID NO:37, assuming an open reading frame having an initiation codon spanning from nucleotide 1 through nucleotide 3 of SEQ ID NO:36 and a stop codon spanning from nucleotide 520 through nucleotide 522 of SEQ ID NO:36. The coding region encoding PFeB7-1s₁₇₃, not including the termination codon, is presented herein as nFeB7-1s₅₁₉, which has the
15 nucleotide sequence SEQ ID NO:39 (the coding strand) and SEQ ID NO:40 (the complementary strand). At the nucleotide level, the first 408 nucleotides of SEQ ID NO:36 were identical to the first 408 nucleotides of coding region of full-length feline B7-1, as represented by GenBank accession number U57755, but the remaining sequences, i.e., nucleotides 409-594, were unique. At the amino acid level, the first 139
20 residues of SEQ ID NO:37 were identical to the first 139 residues of full-length feline B7-1 as represented by PID accession number g2065521, but the remaining sequence, i.e., amino acids 140-173, were unique.

Both full-length and the truncated forms of feline B7-1 transcripts were expressed by spleen and lymph node cells of cats as indicated by RT-PCR of messenger
25 RNA isolated from these cells. RT-PCR reactions were carried out with primers specific for the full-length feline B7-1 using sense primer 5' ACC ACT CCA TTG TGA TCA TG 3', denoted herein as SEQ ID NO:56, and corresponding to nucleotides 293-312 of GenBank accession number U57755; and antisense primer SEQ ID NO:54. RT-PCR reactions were also carried out with primers specific for the truncated form of feline B7-
30 1 using sense primer SEQ ID NO:53; and antisense primer 5' GTC TTG ATC TCA GGG TCA TG 3', denoted herein as SEQ ID NO:57, and corresponding to the reverse

complement of nucleotides 465-484 of SEQ ID NO:36. The RT-PCR reaction amplified fragments of 587 and 484 base pairs, which corresponded to the full-length and truncated forms of feline B7-1.

- When compared to the domain structure of B7-1 from human, mouse and dog,
- 5 the divergence point of the truncated feline B7-1 amino acid sequence, i.e. between amino acid residues 139 and 140 of SEQ ID NO:38, corresponded to the junction between the IgV- and IgC-like domains in the extracellular regions of the human, mouse, and dog molecules. It is likely that, while the soluble form of canine B7-1, as described in Example 2.C., consists of both IgV- and Ig-C like domains, soluble feline B7-1
- 10 consists of only the IgV-like domain. Previous work by Rennert et al., *Int. Immunol.*, Vol. 9, pp. 805-813, 1997, indicated that a fusion protein consisting of the IgV-like domain of human B7-1 did not bind to CTLA4 while a fusion protein consisting of the IgV-like domain of human B7-2 or IgV- and IgC-like domains of either B7-1 or B7-2 bound to CTLA4. It is not known at the present time whether the feline B7-1 protein
- 15 encoded by the truncated transcript can bind to B7 receptors CD28 or CTLA4.

Example 8

This example describes the production of recombinant molecules encoding feline B7-1 and B7-2 proteins, and the expression of these recombinant molecules in mammalian cells.

- 20 A. A recombinant plasmid, denoted herein as pCMV-nFeB7-1₈₇₉, capable of expressing a full length form of B7-1, was produced as follows. Feline B7-1 was cloned from the feline PBMC cDNA library, produced as described in Example 5, by PCR using the feline B7-1 gene-specific primers SEQ ID NO:53 and SEQ ID NO:54. The resulting PCR fragment was first cloned into pCR2.1 (Invitrogen), and then the insert
- 25 was excised with restriction endonucleases *Eco* R1 and *Xho* I. The insert was gel purified and ligated into the HCMV immediate-early transcription control region of the pCMV-Int A plasmid vector, produced as described in Example 3.A., that had been digested by *Eco* RI and *Xho* I and gel purified, to produce recombinant molecule pCMV-nFeB7-1₈₇₉. Insert size and identity were confirmed by restriction digestion,
- 30 PCR, and sequencing.

- B. A recombinant plasmid, denoted herein as pCMV-nFeB7-2₉₉₉, capable of expressing a full length form of feline B7-2, was produced as follows. The complete coding region encoding feline B7-2, i.e., nucleotides 179-1177 of SEQ ID NO:25, was amplified by PCR from full length cDNA nFeB7-2₂₈₃₀ with the following gene specific
- 5 primers: sense primer 5' GCG **GAT CCA CCA TGG GCA TTT GTG ACA GCA C** 3', denoted herein as SEQ ID NO:58, with nucleotides 12-31 corresponding to nucleotides 179-198 of SEQ ID NO:25; and antisense primer 5' GCC **TCG AGT TAA AAA TGT GTA GTA CTT TTG TCG** 3', denoted herein as SEQ ID NO:59, with nucleotides 9-33 corresponding to the reverse complement of nucleotides 1153-1177 of SEQ ID NO:25.
- 10 To facilitate cloning into pCMV-IntA, a *Bam* H1 site (shown in bold) was added to the sense primer SEQ ID NO:58, and an *Xho* I site (shown in bold) was added to the antisense primer SEQ ID NO:59. The PCR-amplified nucleic acid molecule nFeB7-2₉₉₉ was digested with restriction endonucleases *Bam* H1 and *Xho* I. The insert was gel purified and ligated into the HCMV immediate-early transcription control region of the
- 15 pCMV-IntA plasmid vector, produced as described in Example 3.A., that had been digested by *Bam* H1 and *Xho* I and gel purified, to produce recombinant molecule pCMV-nFeB7-2₉₉₉. The insert size and identity were confirmed by restriction digestion, PCR, and sequencing.

- C. Stable recombinant cells individually expressing recombinant plasmids
- 20 pCMV-nFeB7-2₉₉₉ and pCMV-nFeB7-1₈₇₉ were established in mouse L-M (TK-) cell fibroblasts (L cells, available from ATCC) as follows. Briefly, six-well polystyrene tissue culture plates were seeded with approximately 5×10^5 cells /well in 2 ml of DMEM, available from Life Technologies, supplemented with 100 mM L-glutamine, gentamicin, and 10% FBS (L-TCM). Cells were grown to about 80% confluence (about
- 25 18 hr). The recombinant plasmids to be transfected were purified using the 5 Prime to 3 Prime Kit as per the manufacturer's instructions. The recombinant plasmids were linearized with the restriction enzyme *Pvu*I. The plasmid PMLBKTk, available from ATCC, which contains the thymidine kinase gene was also linearized with *Pvu*I. Approximately 2 μ g of recombinant plasmid DNA and 0.2 μ g of PMLBKTk were
- 30 mixed with about 100 μ l OptiMEM medium, available from Life Technologies. About 10 μ l Lipofectamine, available from Life Technologies, was mixed with 100 μ l

OptiMEM. The plasmid mixture was then added to the Lipofectamine mixture and incubated at room temperature for about 45 min. After incubation, about 800 μ l of OptiMEM was added, and the entire mixture was overlaid onto the L cells that had been rinsed with OptiMEM. Cells were incubated for 5 hours at 37° C, 5% CO₂, 95% relative humidity. Approximately 1 ml of L-TCM with 20% FBS was added, and the cells were incubated overnight. The media was changed after about 24 hr. About 72 hr post transfection, the cells were split 1:4 and put into selection L-TCM containing 1 X HAT, available from Sigma. The media was changed every 3-5 days. After several weeks, HAT-resistant colonies were trypsinized using cloning cylinders, and the cells were plated into 24 well plates. The resulting recombinant cells are referred to herein as L-pCMV-nFeB7-2₉₉₉ and L-pCMV-nFeB7-1₈₇₉ respectively. The recombinant cells were then expanded for testing.

Example 9

This example describes the detection of expression of feline B7-2 protein expressed in L-pCMV-nFeB7-2₉₉₉ or feline B7-1 protein expressed in L-pCMV-nFeB7-1₈₇₉.

Recombinant cells L-pCMV-nFeB7-2₉₉₉, and L-pCMV-nFeB7-1₈₇₉, produced as described in Example 8, were tested for surface expression of feline B7-1 or B7-2 by determining if human CTLA4 will bind. Briefly, 1 x 10⁵ (per condition) L-pCMV-nFeB7-2₉₉₉ or L-pCMV-nFeB7-1₈₇₉ cells or, as a negative control, L cells transfected with the empty pCMV vector and the PMLBTKK plasmid (L-CMV) were incubated in phosphate buffered saline (PBS) containing 30% fetal bovine serum (FBS) for about 30 min on ice. The cells were then spun down and treated with the following:

	<u>Primary incubation</u>	<u>Secondary Incubation</u>
25	Human CTLA4/Fc	Mouse anti human IgG Fc FITC

Human CTLA4 Fc chimeric protein, available from R&D systems, Minneapolis, MN, was used at about 20 μ g/ml. Mouse anti human IgG Fc FITC, available from Sigma, was used at about 65 μ g/ml. These reagents were diluted in PBS/5%FBS. All incubations were performed in about 100 μ l for about 1 hr on ice with 3 washes of PBS/5%FBS in between each incubation. Cells were then analyzed on a flow cytometer

(MoFlow Desk Top System, Cytomation, Ft Collins, CO) with the fluorescein gate set at 10^1 . The results are shown below in Table 3.

Table 3. Binding of human CTLA4/Fc chimeric protein to feline B7-2 and B7-1 molecules expressed on L cell transfectants.

5

Cells	% positive
L-CMV	1
L-pCMV-nFeB7-2 ₉₉₉	98
L-pCMV-nFeB7-1 ₈₇₉	84

10 This experiment shows that feline B7-2 and B7-1 expressed on the membrane of L cell transfectants are able to bind to recombinant CTLA4 protein.

Example 10

This example describes the expression of canine B7-1 and B7-2 proteins in mammalian cells. Stable recombinant cells individually expressing recombinant plasmids pCMV-nCaB7-2₁₈₉₇ and pCMV-nCaB7-1₁₃₈₅, produced as described in
 15 Examples 3.A. and 3.B., respectively, were established in mouse L cells by the method described in Example 8.C. The resulting recombinant cells are referred to herein as L-pCMV-nCaB7-2₁₈₉₇ and L-pCMV-nCaB7-1₁₃₈₅, respectively. The recombinant cells were then expanded for testing.

Example 11

20 This example describes the cloning of cDNA encoding *Canis* and *Felis* CTLA4.

Two oligonucleotide primers were made according to conserved regions of human, bovine, rabbit, mouse, and rat CTLA4 gene sequences available in GenBank: sense primer, 5' GTG AAC CTS ACY ATC CAA GG 3', where S was either G or C and Y was either T or C, denoted herein as SEQ ID NO:60; and antisense primer,
 25 5' GCA TTT TCA CAT AGA CCC CTG 3', denoted herein as SEQ ID NO:61. The primers correspond to the positions as shown below:

Database	Accession Number	Nucleotides		Animal
		Sense	Antisense	
gb	X05719	391-410	650-670	mouse
gb	U37121	334-353	593-613	rat
gb	L15006	334-353	593-613	human
gb	X93305	328-347	587-607	cattle
dbj	D49844	382-401	641-661	rabbit

- 5
- A. A 280 nucleotide fragment of canine CTLA4, obtained by PCR using the two primers described above, was used to isolate from the canine PBMC cDNA library, prepared as described in Example 1, a clone (Clone 21), referred to herein as
- 10 nCaCTLA4₁₈₅₆, the coding strand of which was shown to have nucleic acid sequence SEQ ID NO:41. The reverse complement of SEQ ID NO:41 is referred to herein as SEQ ID NO:43. Translation of SEQ ID NO:41 suggests that nucleic acid molecule nCaCTLA4₁₈₅₆ encodes a CTLA4 protein of 223 amino acids, denoted herein as PCaCTLA4₂₂₃, the amino acid sequence of which is presented in SEQ ID NO:42,
- 15 assuming an open reading frame having an initiation codon spanning from nucleotide 60 through nucleotide 62 of SEQ ID NO:41 and a stop codon spanning from nucleotide 729 through nucleotide 731 of SEQ ID NO:41. The coding region encoding PCaCTLA4₂₂₃, not including the termination codon, is presented herein as nCaCTLA4₆₆₉, which has the nucleotide sequence SEQ ID NO:44 (the coding strand) and SEQ ID NO:45 (the
- 20 complementary strand).

- B. A 280 nucleotide fragment of feline CTLA4 obtained by PCR using the two primers described above was used to isolate from the feline PBMC cDNA library, prepared as described in Example 5, a clone (Clone 16), referred to herein as
- 25 nFeCTLA4₁₈₈₃, the coding strand of which was shown to have a nucleic acid sequence SEQ ID NO:46. The reverse complement of SEQ ID NO:46 is referred to herein as SEQ ID NO:48. Translation of SEQ ID NO:46 suggests that nucleic acid molecule nFeCTLA4₁₈₈₃ encodes a CTLA4 protein of 223 amino acids, denoted herein as PFeCTLA4₂₂₃, the amino acid sequence of which is presented in SEQ ID NO:47, assuming an open reading frame having an initiation codon spanning from nucleotide 69
- 30 through nucleotide 71 of SEQ ID NO:46 and a stop codon spanning from nucleotide 738

through nucleotide 740 of SEQ ID NO:46. The coding region encoding PFeCTLA4₂₂₃, not including the termination codon, is presented herein as nFeCTLA4₆₆₉, which has the nucleotide sequence SEQ ID NO:49 (the coding strand) and SEQ ID NO:50 (the complementary strand).

- 5 Sequence analysis was performed with DNAsis™ using the alignment settings of: gap penalty set at 5; number of top diagonals set at 5; fixed gap penalty set at 10; k-tuple set at 2; window size set at 5 and floating gap penalty set at 10. At the amino acid level, PCaCTLA4₂₂₃ shared 88.5%, 88.2%, 87.4%, 76.7%, and 76.2% of identity with the CTLA4 proteins of rabbit, bovine, human, mouse, and rat, respectively.
- 10 PFeCTLA4₂₂₃ shared 88.8%, 87.9%, 86.9%, 77.6%, and 77.1% of identity with the CTLA4 proteins of rabbit, human, bovine, mouse, and rat, respectively. PCaCTLA4₂₂₃ and PFeCTLA4₂₂₃ shared the highest identity (97.2%).

- At the nucleotide level, nCaCTLA4₁₈₅₆ shared 86.7%, 86.7%, 86.3%, 76.1%, and 59.5% identity with the cDNA sequences of human, rabbit, bovine, rat, and mouse
- 15 CTLA4, respectively. Nucleic acid molecule nFeCTLA4₁₈₈₃ shared 88.7%, 86.4%, 86.3%, 77.3% and 57.3 % identity with the cDNA sequences of human, rabbit, bovine, rat, and mouse CTLA4, respectively. Nucleic acid molecules nCaCTLA4₁₈₅₆ and nFeCTLA4₁₈₈₃ shared 89.2% identity.

Example 12

- 20 This example describes the expression in *Pichia* of a canine CTLA4 cDNA fragment, nucleotides 168-542 of SEQ ID NO:41, encoding the extracellular domain of the mature canine CTLA4 protein, i.e., amino acid residues 37-161 of SEQ ID NO: 42. A 375-nucleotide fragment, denoted herein as nCaCTLA4₃₇₅, was isolated from nCaCTLA4₁₈₅₆ by PCR using sense primer 5' GGT **ACG TAG** GGA TGC ATG TGG
- 25 CTC AGC 3' denoted herein as SEQ ID:62, with nucleotides 9-27 corresponding to nucleotides 168-186 of SEQ ID NO:41; and antisense primer 5' CCG **AAT TCT CAG** TCA GAA TCT GGG CAA GGT TC 3', denoted herein as SEQ ID NO:63, with nucleotides 9-32 corresponding to the reverse complement of nucleotides 522-542 of SEQ ID NO:41. To facilitate cloning, an *Sna* BI site (shown in bold) was added to the
- 30 sense primer and an *Eco* RI site (shown in bold) was added to the antisense primer. The PCR fragment was digested with restriction endonucleases *Sna* B I and *Eco* RI, gel

purified and ligated into pPIC9K plasmid vector, available from Invitrogen, that had been digested by *Sna* RI and *Eco* RI and gel purified to produce recombinant molecule pPIC9K-nCaCTLA4₃₇₅. The insert in the recombinant plasmid was verified by DNA sequencing. After linearization, the plasmid was used to transform *Pichia* strain GS115, available in kit form from Invitrogen, by electroporation. Colonies containing putative high copy number of the plasmid was selected on plates with G418. Colonies resistant to at least 200µg/ml of G418 were selected for expression by induction with 0.5% methanol in BMM, available from Invitrogen, according to manufacturer's instructions. Recombinant protein was isolated using a Sepharose Q high-trap column, available from Pharmacia Biotech, Inc., Piscataway, NJ. The identity of the protein was verified by N-terminal sequencing.

Example 13

This example describes the ability of a recombinant canine CTLA4 protein expressed in *Pichia* to bind to canine B7-1 or B7-2, expressed on the surface of mouse L-cells.

Recombinant canine CTLA4 protein, produced in *Pichia*, was tested for its ability to bind to canine B7 molecules, as follows. Briefly, 1×10^5 (per condition) L-pCMV-nCaB7-2₁₈₉₇ or L-pCMV-nCaB7-1₁₃₈₅, produced as described in Example 10, were incubated in phosphate buffered saline (PBS) containing 30% fetal bovine serum (FBS) for about 30 min on ice. The cells were then spun down and treated in one of the following manners:

Condition	Primary Incubation	Secondary Incubation	Tertiary Incubation
1	PBS/5% FBS	PBS/5 % FBS	Rabbit anti goat IgG FITC
2	PBS/5% FBS	Goat anti human CTLA4	Rabbit anti goat IgG FITC
3	Canine CTLA4	Goat anti human CTLA4	Rabbit anti goat IgG FITC

Polyclonal rabbit anti goat IgG FITC, available from Southern Biotechnologies, Birmingham, AL, was used at approximately 10 µg/ml. Polyclonal goat anti human CTLA4, available from R & D systems, was used at approximately 2 µg/ml. Canine CTLA4 expressed in *Pichia* was used at 60-600 µg/ml. All reagents were diluted in PBS/5%FBS.

All incubations were performed in about 100 μ l for about 1 hr on ice with 3 washes of PBS/5%FBS in between each incubation. Cells were then analyzed on a flow cytometer as described in Example 9. The results are shown below in Tables 4 and 5.

5 Table 4. Binding of canine CTLA4 expressed in *Pichia* to canine B7-2 expressed on L cell transfectants.

Condition	% positive cells
1	1
2	1
3	89

10 Table 5. Binding of canine CTLA4 expressed in *Pichia* to canine B7-1 expressed on L cell transfectants.

Condition	% positive cells
1	4
2	2
3	63

15 This experiment shows that recombinant canine CTLA-4 protein produced in *Pichia* is able to bind to canine B7-2 and B7-1 expressed on the membrane of recombinant L cells.

Example 14

20 This example describes the production of mouse polyclonal sera to canine B7-2 expressed on L-pCMV-nCaB7-2₁₈₉₇ and expression in *Pichia* of soluble canine B7-2.

A. Mouse antibodies specifically reactive with canine B7-2 were produced in mice as follows: Briefly, Balb/c mice were immunized with about 10^6 L-pCMV-nCaB7-2₁₈₉₇ recombinant cells, prepared as described in Example 10. The mice were boosted
25 after about 4 weeks with about 1×10^6 L-pCMV-nCaB7-2₁₈₉₇ recombinant cells. Serum isolated from the mice was screened by flow cytometry for positive reactivity on L-pCMV-nCaB7-2₁₈₉₇ recombinant cells.

The sera was negative on L-CMV cells (described in Example 9) and on L-pCMV-nCaB7-1₁₃₈₅ recombinant cells, prepared as described in Example 10. This
30 suggests that the sera was not cross-reactive with canine B7-1.

B. A 780-nucleotide canine B7-2 cDNA fragment, i.e., nucleotides 70-849 of SEQ ID NO. 16, encoding the mature portion of soluble canine B7-2 protein, i.e., residues 22-280 of SEQ ID NO. 17, was isolated by PCR from nCaB7-2s₁₇₉₅ using sense primer 5' GGT **ACG TAG** GTG CTG CTT CCA TGA AGA G 3', denoted herein as SEQ ID NO:64, with nucleotides 9-28 corresponding to nucleotides 70-89 of SEQ ID NO:16; and antisense primer, CCC **CTA GGT** TAA AAC TGT GTA GTA CTG TTG TCG CC-3', denoted herein as SEQ ID NO: 65, with nucleotides 9-35 corresponding to the reverse complement of nucleotides 823-849 of SEQ ID NO:16. To facilitate cloning, an *Sna* BI site (shown in bold) was added to the sense primer and an *Avr* II site (shown in bold) was added to the antisense primer. The PCR fragment was digested with restriction endonucleases *Sna* B1 and *Avr* II, gel purified and ligated into pPIC9K plasmid vector that had been digested by *Sna* R1 and *Avr* II and gel purified to produce recombinant molecule pPIC9K-nCaB7-2s₇₈₀. The insert in the recombinant plasmid was verified by DNA sequencing. After linearization, the plasmid was used to transform GS115 strain of *Pichia*. Secreted protein in the supernatant from G418 resistant colonies, produced as described in Example 12, specifically reacted in immunoblots and ELISA with polyclonal antibodies produced in mice immunized with canine B7-2 recombinant cell pCMV-nCaB7-2₁₈₉₇, produced as described in Example 14.A., above.

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.

What is claimed is:

1. An isolated nucleic acid molecule selected from the group consisting of: a nucleic acid molecule having a nucleic acid sequence that is at least about 80 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, or a fragment thereof having at least about 12 nucleotides; a nucleic acid molecule consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, and SEQ ID NO:40; and a nucleic acid molecule having a nucleic acid sequence that is at least about 90 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, or a fragment thereof having at least about 12 nucleotides.

2. An isolated nucleic acid molecule selected from the group consisting of:
(a) a nucleic acid molecule having a nucleic acid sequence encoding a B7 protein selected from the group consisting of a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:2, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:2, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:7, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:7, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:12, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:12, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein comprising an epitope of said protein having an amino acid sequence that is at least

about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:26, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:26, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:34, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:34, and a protein having amino acid sequence SEQ ID NO:37;

10 (b) a nucleic acid molecule comprising a complement of any of said nucleic acid sequences set forth in (a);

(c) a nucleic acid molecule having a nucleic acid sequence encoding a CTLA4 protein selected from the group consisting of: a protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; and a protein comprising an epitope of said protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; and

20 (d) a nucleic acid molecule comprising a complement of any of said nucleic acid sequences set forth in (c); wherein said B7 protein elicits an immune response against a naturally-occurring B7 protein, and wherein said CTLA4 protein elicits an immune response against a naturally-occurring CTLA4 protein.

3. An isolated nucleic acid molecule selected from the group consisting of a nucleic acid molecule that encodes a naturally-occurring soluble mammalian B7 protein and a nucleic acid molecule comprising a complement of said nucleic acid molecule that encodes said protein.

4. An isolated protein selected from the group consisting of:

(a) an isolated protein comprising a B7 protein selected from the group consisting of a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:2, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to

the amino acid sequence SEQ ID NO:2, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:7, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:7, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:12, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:12, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:26, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:26, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:34, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:34, and a protein consisting of amino acid sequence SEQ ID NO:37, wherein said B7 protein elicits an immune response against a naturally-occurring B7 protein; and

(b) an isolated protein comprising a CTLA4 protein selected from the group consisting of: a protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; and a protein comprising an epitope of said protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; wherein said CTLA4 protein elicits an immune response against a naturally-occurring CTLA4 protein.

5. An isolated naturally-occurring soluble mammalian B7 protein.
6. A therapeutic composition that, when administered to an animal, regulates T cell mediated immune responses in said animal, said therapeutic composition

comprising a therapeutic compound selected from the group consisting of: an isolated protein comprising a B7 protein, wherein said B7 protein is selected from the group consisting of a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:2, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:2, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:7, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:7, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:12, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:12, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:26, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:26, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:34, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:34, a protein having amino acid sequence SEQ ID NO:37, an isolated naturally-occurring soluble B7 protein; a mimotope of any of said B7 proteins; a multimeric form of any of said B7 proteins; an isolated protein comprising a CTLA4 protein selected from the group consisting of: a protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; and a protein comprising an epitope of said protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; a mimotope of any of said CTLA4 proteins; a multimeric

form of any of said CTLA4 proteins; an isolated nucleic acid molecule selected from the group consisting of: a nucleic acid molecule having a nucleic acid sequence that is at least about 80 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:35; a nucleic acid molecule consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, and SEQ ID NO:40; and a nucleic acid molecule having a nucleic acid sequence that is at least about 90 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, and SEQ ID NO:50; an isolated antibody that selectively binds to any of said B7 proteins; an inhibitor of B7 protein activity identified by its ability to inhibit the activity of any of said B7 proteins; an isolated antibody that selectively binds to any of said CTLA4 proteins; and an inhibitor of CTLA4 protein activity identified by its ability to inhibit the activity of any of said CTLA4 proteins.

7. A method to regulate T cell mediated immune responses in an animal comprising administering to the animal a therapeutic composition comprising a therapeutic compound selected from the group consisting of: an isolated protein comprising a B7 protein, wherein said B7 protein is selected from the group consisting of a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:2, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:2, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:7, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:7, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:12, a protein comprising an epitope of said protein having an

amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:12, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:17, a protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:26, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 60 percent identical to the amino acid sequence SEQ ID NO:26, a protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:34, a protein comprising an epitope of said protein having an amino acid sequence that is at least about 80 percent identical to the amino acid sequence SEQ ID NO:34, a protein having amino acid sequence SEQ ID NO:37, an isolated naturally-occurring soluble B7 protein; a mimotope of any of said B7 proteins; a multimeric form of any of said B7 proteins; an isolated protein comprising a CTLA4 protein selected from the group consisting of: a protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; and a protein comprising an epitope of said protein having an amino acid sequence that is at least about 90 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; a mimotope of any of said CTLA4 proteins; a multimeric form of any of said CTLA4 proteins; an isolated nucleic acid molecule selected from the group consisting of: a nucleic acid molecule having a nucleic acid sequence that is at least about 80 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:35; a nucleic acid molecule consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, and SEQ ID NO:40; and a nucleic acid molecule having a nucleic acid sequence that is at least about 90 percent identical to

a nucleic acid sequence selected from the group consisting of SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, and SEQ ID NO:50; an isolated antibody that selectively binds to any of said B7 proteins; an inhibitor of B7 protein activity identified by its ability to inhibit the activity of any of said B7 proteins; an isolated antibody that selectively binds to any of said CTLA4 proteins; and an inhibitor of CTLA4 protein activity identified by its ability to inhibit the activity of any of said CTLA4 proteins.

8. A method to produce a protein selected from the group consisting of a B7 protein and a CTLA4 protein, said method comprising culturing a cell capable of expressing said protein, said protein being encoded by a nucleic acid molecule selected from the group consisting of: a nucleic acid molecule having a nucleic acid sequence that is at least about 80 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, or a fragment thereof having at least about 18 nucleotides, wherein said fragment encodes an epitope; a nucleic acid molecule consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, and SEQ ID NO:40; a nucleic acid molecule having a nucleic acid sequence that is at least about 90 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, or a fragment thereof having at least about 18 nucleotides, wherein said fragment encodes an epitope; and a nucleic acid molecule that encodes a naturally-occurring soluble mammalian B7 protein.

9. A method to identify a compound capable of regulating T cell mediated immune responses in an animal, said method comprising:

(a) contacting an isolated protein selected from the group consisting of a B7 protein and a CTLA4 protein with a putative inhibitory compound under conditions in which, in the absence of said compound, said protein has T cell stimulating activity,

wherein said B7 protein is selected from the group consisting of: a protein having an amino acid sequence that is at least about 80 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:12 and SEQ ID NO:34; and a protein having an amino acid sequence that is at least about 60 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:17 and SEQ ID NO:26, and wherein said CTLA4 protein is selected from the group consisting of a protein having an amino acid sequence that is at least about 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:47; and

10 (b) determining if said putative inhibitory compound inhibits said activity.

10. The invention of Claims 1,2, or 6-8, wherein said nucleic acid molecule comprises a nucleic acid sequence that encodes a protein selected from the group consisting of a B7 protein and a CTLA4 protein.

11. The invention of Claims 1,2, or 6-8, wherein said nucleic acid molecule encodes a protein that elicits an immune response against a protein selected from the group consisting of a naturally-occurring B7 protein and a naturally-occurring CTLA4 protein.

12. The invention of Claims 1,2, or 6-8, wherein said nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid molecule selected from the group consisting of nCaB7-1₂₈₃₀, nCaB7-1₁₃₈₅, nCaB7-1₉₁₂, nCaB7-2₁₈₉₇, nCaB7-2₉₈₇, nCaB7-1s₁₀₂₄, nCaB7-1s₇₀₅, nCaB7-2s₁₇₉₅, nCaB7-2s₈₄₀, nFeB7-2₂₈₃₀, nFeB7-2₉₉₆, nCaB7-1₈₁₀, nCaB7-2₉₂₁, nCaB7-1s₆₀₃, nCaB7-2s₇₇₄, nFeB7-2₉₁₈, nFeB7-2₅₀₉, nFeB7-2s₃₅₉, nCaCTLA4₁₈₅₆, nCaCTLA4₆₇₂, nFeCTLA4₁₈₈₃, and nFeCTLA4₄₆₇₂; and a nucleic acid molecule consisting of a nucleic acid molecule selected from the group consisting of nFeB7-1s₅₉₄ and nFeB7-1s₅₁₉.

13. The invention of Claims 1,2, or 6-8, wherein said nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence that encodes a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12, SEQ ID NO:17, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:42, and SEQ ID NO:47; a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule encoding a protein

- having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12, SEQ ID NO:17, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:42, and SEQ ID NO:47; a nucleic acid molecule consisting of a nucleic acid sequence that encodes a protein having amino acid sequence SEQ ID NO:37; and a nucleic acid molecule consisting of an allelic variant of a nucleic acid molecule that encodes a protein having amino acid sequence SEQ ID NO:37.

14. The invention of Claims 1,2, or 6-8, wherein said nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:49, and SEQ ID NO:50; and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic acid sequences.

15. The invention of Claims 1-3 or 6-8, wherein said nucleic acid molecule is selected from the group consisting of: a nucleic acid molecule consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:39, and SEQ ID NO:40. and a nucleic acid molecule consisting of an allelic variant of a nucleic acid molecule consisting of any of said nucleic acid sequences.

16. The invention of Claims 1-3 or 6-8, wherein said nucleic acid molecule comprises an oligonucleotide.

17. A recombinant molecule comprising a nucleic acid molecule as set forth in Claims 1-3 or 6-8, operatively linked to a transcription control sequence.

18. A recombinant virus comprising a nucleic acid molecule as set forth in Claims 1-3 or 6-8.

19. A recombinant cell comprising a nucleic acid molecule as set forth in Claims 1-3 or 6-8.

20. The nucleic acid molecule of Claim 3, wherein said naturally-occurring soluble mammalian B7 protein is selected from the group consisting of a naturally-occurring soluble mammalian B7-1 protein and a naturally-occurring soluble mammalian B7-2 protein.

5 21. The nucleic acid molecule of Claim 3, wherein said nucleic acid molecule comprises a nucleic acid sequence encoding a naturally-occurring soluble B7-2 protein having extracellular and intracellular domains but lacking at least a portion of a transmembrane domain sufficient to produce a soluble protein upon translation of said nucleic acid molecule in a suitable host cell.

10 22. The nucleic acid molecule of Claim 3, wherein said nucleic acid molecule comprises a nucleic acid sequence encoding a naturally-occurring soluble B7-1 protein having an extracellular domain but lacking at least a portion of transmembrane and intracellular domains sufficient to produce a soluble protein upon translation of said nucleic acid molecule in a suitable host cell.

15 23. The nucleic acid molecule of Claim 3, wherein said naturally-occurring soluble mammalian B7 protein is capable of binding to a protein selected from the group consisting of CD28 and CTLA4.

20 24. The nucleic acid molecule of Claim 3, wherein said nucleic acid molecule comprises a nucleic acid sequence encoding a naturally-occurring soluble feline B7-1 protein having an extracellular domain but lacking at least a portion of transmembrane and intracellular domains sufficient to produce a soluble protein upon translation of said nucleic acid molecule in a suitable host cell, wherein said extracellular domain comprises an IgV-like domain, but lacks an IgC-like domain.

25 25. The nucleic acid molecule of Claim 3, wherein said naturally-occurring soluble mammalian B7 protein is capable of delivering a co-stimulatory signal to a helper T cell sufficient to stimulate cytokine secretion by said helper T cell.

26. The nucleic acid molecule of Claim 1-3 or 6-8, wherein said nucleic acid molecule is selected from the group consisting of a canine nucleic acid molecule and a feline nucleic acid molecule.

27. The invention of Claims 4 or 6-9, wherein said protein, when administered to an animal, elicits an immune response against a protein selected from the group consisting of a B7-1 protein, a B7-2 protein and a CTLA4 protein.

28. The invention of Claims 4 or 6-9, wherein said protein is selected from the group consisting of: a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12, SEQ ID NO:17, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:42, and SEQ ID NO:47; a protein encoded by an allelic variant of a nucleic acid molecule encoding a protein selected from the group consisting of SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12, SEQ ID NO:17, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:42, and SEQ ID NO:47; a protein consisting of amino acid sequence SEQ ID NO:37; and a protein encoded by an allelic variant of a nucleic acid molecule encoding a protein consisting of amino acid sequence SEQ ID NO:37.

29. An isolated antibody that selectively binds to a protein as set forth in Claims 4-9.

30. The protein of Claim 5, wherein said protein is selected from the group consisting of a naturally-occurring soluble mammalian B7-1 protein and a naturally-occurring soluble mammalian B7-2 protein.

31. The protein of Claim 4-9, wherein said protein is selected from the group consisting of a canine protein and a feline protein.

32. The protein of Claim 5, wherein said protein comprises an isolated naturally-occurring soluble B7-2 protein comprising extracellular and intracellular domains but lacking at least a portion of a transmembrane domain sufficient to be a soluble B7-2 protein.

33. The protein of Claim 5, wherein said protein comprises an isolated naturally-occurring soluble B7-1 protein comprising an extracellular domain but lacking at least a portion of transmembrane and intracellular domains sufficient to produce a soluble B7-1 protein.

34. The protein of Claim 5, wherein said protein comprises a naturally-occurring soluble feline B7-1 protein having an extracellular domain but lacking at least a portion of transmembrane and intracellular domains sufficient to produce a soluble

protein upon translation of said nucleic acid molecule in a suitable host cell, wherein said extracellular domain comprises an IgV-like domain, but lacks an IgC-like domain.

35. The protein of Claim 5, wherein said protein is capable of binding to a protein selected from the group consisting of CD28 and CTLA4.

5 36. The protein of Claim 5, wherein said protein is capable of delivering a co-stimulatory signal to a helper T cell sufficient to stimulate cytokine secretion by said helper T cell.

37. The invention of Claim 6 or Claim 7, wherein said composition further comprises a component selected from the group consisting of an excipient, an adjuvant
10 and a carrier.

38. The invention of Claim 6 or Claim 7, wherein said therapeutic compound is selected from the group consisting of a naked nucleic acid vaccine and a recombinant cell vaccine.

39. The method of Claim 7, wherein said animal is selected from the group
15 consisting of canines and felines.



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: C12N 15/12, C07K 14/705, C12N 15/86, 5/10, A61K 49/00, C07K 16/28	A3	(11) International Publication Number: WO 99/47558 (43) International Publication Date: 23 September 1999 (23.09.99)
(21) International Application Number: PCT/US99/06187 (22) International Filing Date: 19 March 1999 (19.03.99) (30) Priority Data: 60/078,765 19 March 1998 (19.03.98) US 09/062,597 17 April 1998 (17.04.98) US (71) Applicant (for all designated States except US): HESKA CORPORATION [US/US]; 1613 Prospect Parkway, Fort Collins, CO 80525 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): SIM, Gek-Kee [US/US]; 3622 Terry Point Drive, Fort Collins, CO 80524 (US). YANG, Shumin [CN/US]; 2624 Shavano Court, Fort Collins, CO 80525 (US). SELLINS, Karen, S. [US/US]; 1919 Enchantment Drive, Fort Collins, CO 80525 (US). (74) Agents: HANLEY, Elizabeth, A. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).	(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 23 December 1999 (23.12.99)	
(54) Title: T CELL COSTIMULATORY PROTEINS, SEQUENCES AND USES THEREOF		
(57) Abstract The present invention relates to B7 proteins; to B7 nucleic acid molecules, including those that encode such B7 proteins; to antibodies raised against such B7 proteins; and to therapeutic compounds that regulate B7 function. The present invention also includes methods to identify and obtain such proteins, nucleic acid molecules, antibodies, and inhibitory compounds. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to regulate an immune response in an animal.		

**DECLARATION, PETITION AND POWER OF ATTORNEY
FOR PATENT APPLICATION**

(Check one):

- ☐ Declaration Submitted with Initial Filing
☒ Declaration Submitted after Initial Filing

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

T CELL COSTIMULATORY PROTEINS, SEQUENCES AND USES THEREOF

the specification of which (check one):

☐ is attached hereto.

OR

☒ was filed on 19 September 2000 as United States Application Number
09/646,561

☐ and was amended by PCT Article 19 Amendment on _____
(if applicable),

☒ and was amended by PCT Article 34 Amendment on _____
(if applicable).

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby state that I have reviewed and understood the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

PRIORITY CLAIM

(Check one):

- ☐ no such applications have been filed.
- ☒ such applications have been filed as follows

1) FOREIGN PRIORITY CLAIM: I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (dd/mm/yyyy)	Priority Not Claimed	Certified Copy Attached	
				Yes	No
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

- ☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto.

2) PROVISIONAL PRIORITY CLAIM: I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

Provisional Application Number(s)	Filing Date (dd/mm/yyyy)
60/078,765	19 March 1998 (19.03.98)

- ☐ Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.

3) U.S./PCT PRIORITY CLAIM: I hereby claim the benefit under Title 35, United States Code, §120 of any United States application or §365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (dd/mm/yyyy)	Parent Patent Number (if applicable)
09/062,597		17 April 1998 (17.04.98)	
	PCT/US99/06187	19 March 1999 (19.03.99)	

- ☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto.

**DECLARATION, PETITION AND POWER OF ATTORNEY
FOR PATENT APPLICATION**

(Check one):

- ☐ Declaration Submitted with Initial Filing
☒ Declaration Submitted after Initial Filing

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

T CELL COSTIMULATORY PROTEINS, SEQUENCES AND USES THEREOF

the specification of which (check one):

☐ is attached hereto.

OR

☒ was filed on **19 September 2000** as United States Application Number
09/646,561

☐ and was amended by PCT Article 19 Amendment on _____
(if applicable),

☐ and was amended by PCT Article 34 Amendment on _____
(if applicable).

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby state that I have reviewed and understood the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

PRIORITY CLAIM

(Check one):

- ☐ no such applications have been filed.
- ☒ such applications have been filed as follows

1) FOREIGN PRIORITY CLAIM: I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (dd/mm/yyyy)	Priority Not Claimed	Certified Copy Attached	
				Yes	No
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto.

2) PROVISIONAL PRIORITY CLAIM: I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

Provisional Application Number(s)	Filing Date (dd/mm/yyyy)
60/078,765	19 March 1998 (19.03.98)

☐ Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.

3) U.S./PCT PRIORITY CLAIM: I hereby claim the benefit under Title 35, United States Code, §120 of any United States application or §365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (dd/mm/yyyy)	Parent Patent Number (if applicable)
09/062,597		17 April 1998 (17.04.98)	
	PCT/US99/06187	19 March 1999 (19.03.99)	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto.

POWER OF ATTORNEY:

As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

James E. Cockfield	Reg. No. 19,162	Nicholas P. Triano III	Reg. No. 36,397
Thomas V. Smurzynski	Reg. No. 24,798	Peter C. Lauro	Reg. No. 32,360
Ralph A. Loren	Reg. No. 29,325	DeAnn F. Smith	Reg. No. 36,683
Giulio A. DeConti, Jr.	Reg. No. 31,503	William D. DeVaul	Reg. No. 42,483
Ann Lamport Hammitte	Reg. No. 34,858	David J. Ridders	Reg. No. 43,882
Elizabeth A. Hanley	Reg. No. 33,505	Chi Suk Kim	Reg. No. 42,728
Amy E. Mandragouras	Reg. No. 36,207	Maria Laccotripe Zacharakis	Limited Recognition
Anthony A. Laurentano	Reg. No. 38,220		Under 37 C.F.R. § 10.9(b)
Jane E. Remillard	Reg. No. 38,872	Debra J. Milasincic	Reg. No. 46,931
Jeremiah Lynch	Reg. No. 17,425	David R. Burns	Reg. No. 46,590
Kevin J. Canning	Reg. No. 35,470	Sean D. Detweiler	Reg. No. 42,482
Jeanne M. DiGiorgio	Reg. No. 41,710	Peter S. Stecher	Reg. No. P47,259
Megan E. Williams	Reg. No. 43,270	Adam M. Goodman	Reg. No. 43,640

Send Correspondence to:

Elizabeth A. Hanley, Lahive & Cockfield, LLP, 28 State Street, Boston, Massachusetts 02109, United States of America

Direct Telephone Calls to: (name and telephone number)

Elizabeth A. Hanley, (617) 227-7400

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Gek-Kee SIM	
Inventor's signature	Date
Residence 3622 Terry Point Drive, Fort Collins, Colorado 80524, United States of America	
Citizenship United States of America	
Post Office Address (if different)	

POWER OF ATTORNEY:

As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

James E. Cockfield	Reg. No. <u>19,162</u>	Nicholas P. Triano III	Reg. No. <u>36,397</u>
Thomas V. Smurzynski	Reg. No. <u>24,798</u>	Peter C. Lauro	Reg. No. <u>32,360</u>
Ralph A. Loren	Reg. No. <u>29,325</u>	DeAnn F. Smith	Reg. No. <u>36,683</u>
Giulio A. DeConti, Jr.	Reg. No. <u>31,503</u>	William D. DeVaul	Reg. No. <u>42,483</u>
Ann Lamport Hammitte	Reg. No. <u>34,858</u>	David J. Ridders	Reg. No. <u>43,882</u>
Elizabeth A. Hanley	Reg. No. <u>33,505</u>	Chi Suk Kim	Reg. No. <u>42,728</u>
Amy E. Mandragouras	Reg. No. <u>36,207</u>	Maria Laccotripe Zacharakis	Limited Recognition
Anthony A. Laurentano	Reg. No. <u>38,220</u>		Under 37 C.F.R. § 10.9(b)
Jane E. Remillard	Reg. No. <u>38,872</u>	Debra J. Milasincic	Reg. No. <u>46,931</u>
Jeremiah Lynch	Reg. No. <u>17,425</u>	David R. Burns	Reg. No. <u>46,590</u>
Kevin J. Canning	Reg. No. <u>35,470</u>	Sean D. Detweiler	Reg. No. <u>42,482</u>
Jeanne M. DiGiorgio	Reg. No. <u>41,710</u>	Peter S. Stecher	Reg. No. <u>P47,259</u>
Megan E. Williams	Reg. No. <u>43,270</u>	Adam M. Goodmann	Reg. No. <u>43,640</u>

Send Correspondence to:

Elizabeth A. Hanley, Lahive & Cockfield, LLP, 28 State Street, Boston, Massachusetts 02109, United States of America

Direct Telephone Calls to: (name and telephone number)

Elizabeth A. Hanley, (617) 227-7400

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Gek-Kee SIM	
Inventor's signature	Date
Residence 3622 Terry Point Drive, Fort Collins, Colorado 80524, United States of America	
Citizenship United States of America	
Post Office Address (if different)	

100
100

Full name of sole or first inventor Shumin YANG	
Inventor's signature <i>Shumin</i>	Date 12/12/00
Residence 3353 Alma St., #250, Palo Alto, CA 94306, USA (SY) 2624 Shavano Court, Fort Collins, Colorado 80525, United States of America	
Citizenship China USA (SY)	
Post Office Address (if different)	

Full name of sole or first inventor Karen S. SELLINS	
Inventor's signature	Date
Residence 1919 Enchantment Drive, Fort Collins, Colorado 80525, United States of America	
Citizenship United States of America	
Post Office Address (if different)	

Full name of sole or first inventor Shumin YANG	
Inventor's signature	Date
Residence 2624 Shavano Court, Fort Collins, Colorado 80525, United States of America	
Citizenship China	
Post Office Address (if different)	

Full name of sole or first inventor Karen S. SELLINS	
Inventor's signature <i>Karen S. Sellins</i>	Date <i>12/7/00</i>
Residence 1919 Enchantment Drive, Fort Collins, Colorado 80525, United States of America CO	
Citizenship United States of America	
Post Office Address (if different)	

WO 99/47558

SEQUENCE LISTING

<110> Sim, Gek-Kee
Yang, Shumin
Sellins, Karen S.

<120> NOVEL FORMS OF T CELL COSTIMULATORY PROTEINS, NUCLEIC
ACID MOLECULES, AND USES THEREOF

<130> IM-1-C1-PCT

<140> not yet assigned

<141> 1999-03-19

<150> 60/078,765

<151> 1998-03-19

<150> 09/062,597

<151> 1998-04-17

<160> 65

<170> PatentIn Ver. 2.0

<210> 1

<211> 2830

<212> DNA

<213> Canis familiaris

<220>

<221> CDS

<222> (337)..(1248)

<400> 1

```
gtgctttgtc ctagccacac tctctgaggt ggctgacaaa aaggacagc agaaccagct 60
tctcaagtt atacataaca tctacacatc cctgctttg acttaaatac tgctggtaat 120
gaacatcagc tagatcttcc agcgagtaaa aggaagttgg aaaggggatt gcctctggta 180
tatcacccaa agaaaagctg agcaacttgc cattattttg gagacagcaa gaaaggaaca 240
tctcagaact ggggcctcat cctttgacgt tttgttttgt tttgttctaa cacaagaaaa 300
aaaaaaaaga ggagttatcc ttcagcagca gaagcc atg gat tac aca gcg aag 354
Met Asp Tyr Thr Ala Lys
```

tgg aga aca cca cca ctc aaa cac cca tat ctc aag gtc tct caq ctc 402
 Trp Arg Thr Pro Pro Leu Lys His Pro Tyr Leu Lys Val Ser Gln Leu
 10 15 20

ttg gtg cta gct agt ctc ttt tac ttc tgt tca ggc atc atc cag gtg 450
 Leu Val Leu Ala Ser Leu Phe Tyr Phe Cys Ser Gly Ile Ile Gln Val
 25 30 35

aac aag aca gtg aaa gaa gta gca gta ctg tcc tgt gat tac aac att 498
 Asn Lys Thr Val Lys Glu Val Ala Val Leu Ser Cys Asp Tyr Asn Ile
 40 45 50

tcc act aca gaa ctg atg aaa gtt cga atc tat tgg caa aag gat gat 546
 Ser Thr Thr Glu Leu Met Lys Val Arg Ile Tyr Trp Gln Lys Asp Asp
 55 60 65 70

gaa gtg gtg ctg gct gtc aca tct gga caa acg aaa gtg tgg tcc aag 594
 Glu Val Val Leu Ala Val Thr Ser Gly Gln Thr Lys Val Trp Ser Lys
 75 80 85

tat gag aat cgc acc ttt gct gac ttc acc aat aac ctc tcc atc gtg 642
 Tyr Glu Asn Arg Thr Phe Ala Asp Phe Thr Asn Asn Leu Ser Ile Val
 90 95 100

att atg gct ctg cgc ctg tca gac aat ggc aaa tac acc tgt atc gtt 690
 Ile Met Ala Leu Arg Leu Ser Asp Asn Gly Lys Tyr Thr Cys Ile Val
 105 110 115

caa aag act gaa aaa agg tct tac aaa gtg aaa cac atg act tcg gtg 738
 Gln Lys Thr Glu Lys Arg Ser Tyr Lys Val Lys His Met Thr Ser Val
 120 125 130

atg tta ttg gtc aga gct gac ttc cct gtc cct agt ata act gac ctt 786
 Met Leu Leu Val Arg Ala Asp Phe Pro Val Pro Ser Ile Thr Asp Leu
 135 140 145 150

gga aat cca tcc cat gac atc aaa agg ata atg tgt tca acc tct gga 834
 Gly Asn Pro Ser His Asp Ile Lys Arg Ile Met Cys Ser Thr Ser Gly
 155 160 165

ggt ttt cca aag cct cac ctc tcc tgg tgg gaa aat gaa gaa gaa ttg 882
 Gly Phe Pro Lys Pro His Leu Ser Trp Trp Glu Asn Glu Glu Glu Leu
 170 175 180

aat gct gcc aac aca aca gtt tcc caa gac ccg gac act gag ttg tac 930
 Asn Ala Ala Asn Thr Thr Val Ser Gln Asp Pro Asp Thr Glu Leu Tyr
 185 190 195

act att agt agt gaa ctg gat ttc aat ata aca agc aac cat agc ttt 978
 Thr Ile Ser Ser Glu Leu Asp Phe Asn Ile Thr Ser Asn His Ser Phe
 200 205 210

gtg tgt ctt gtc aag tat gga gac tta aca gta tca cag atc ttc aac 1026
 Val Cys Leu Val Lys Tyr Gly Asp Leu Thr Val Ser Gln Ile Phe Asn
 215 220 225 230

tgg caa aaa tca gtc gag cca cac cct ccc aat aac cag caa cag ctc 1074
 Trp Gln Lys Ser Val Glu Pro His Pro Pro Asn Asn Gln Gln Gln Leu
 235 240 245

tgg gtc atc ctg atc tta gta gta agt ggt gtg att gct gtg atc act 1122
 Trp Val Ile Leu Ile Leu Val Val Ser Gly Val Ile Ala Val Ile Thr
 250 255 260

gcc att aca gga ggc tgc cta gcc cac aga tct gct gca aga tgg aga 1170
 Ala Ile Thr Gly Gly Cys Leu Ala His Arg Ser Ala Ala Arg Trp Arg
 265 270 275

cag aga aat agg aac aaa gag gac atg gac ctg gaa aag atg tcc cct 1218
 Gln Arg Asn Arg Asn Lys Glu Asp Met Asp Leu Glu Lys Met Ser Pro
 280 285 290

ata aac ata gga tct gcc caa gca tct gta tgagcagaac atctggaggt 1268
 Ile Asn Ile Gly Ser Ala Gln Ala Ser Val
 295 300

cccacctcca tcttagattg acctcatctt tgaatttctt cagatggcca ggattatccc 1328

accttgcaact tcatgcatct gttctctagg agcctgttca tttcagtggc cctgcagaaa 1388

gtgaccagag gaatatggtg gggacataag tagctctctg gtagccttgg tcaaagaatt 1448

gttcaggcct gggaagagac attcggaaaa tacttgtctc attaatgaca aggacatcaa 1508

ggcctagggg gtgacctgaa tgataaaggt ctgagctaga acccagattt cctgtctcgg 1568

gtgctctttt ccatcagtag tccggctctg tgctattaac tgggtgtgtac aggtgtacac 1628

accagtcaaa atgcttcttg aaaaagagta tgtccaatgt caggtcaact tcagagactt 1688

catctgatgc aacactagaa ggttttgtgt tgctgtcaaa agcaatctga tgctaattgtg 1748

tggtagtatg atggtatata taccaatatg agaattgatg aaaaattact ggggtttact 1808

cagtatctca tctttcattg tgttctctct tgttgcttct ccacttctcc atcaggttct 1868

ggagaaagta gatctatcca aaactaatat ctgctgacat gtaagatgaa tgacttatat 1928
 acctcaaagc gatagtcacg ttggagaggg ataggttggt ttagagagtc acatccact 1988
 ggttcatatt ggactgataa tctccttaat ggctttatgc tagtttaaac tcatttataa 2048
 aacatgagaa agttctcatt taaaatgaga taggttttaa ttgtatatta ctaaacagat 2108
 attactaaac agtagctgtc cttagaattt gattgaggtg atgaaaatag cattccatag 2168
 ggtttcccta gattcctcaa gttgctcttc ctcttggtg tttctgatcc ttctgacatc 2228
 agcagagaat taaaaataaa gaagtggcca actgccgttc ctgtgtcact tactcatgat 2288
 tcttttctct gaagttgtct tcataactc agtggacctg gaggtagacc tgactggagg 2348
 aatcagacat tctcatttga aaatttgacg ttggacagca agttggccaa gtttctcaca 2408
 tgtagctggg ttacaatgt ttaattttgg cagctatcaa gggaccagat tatgctatat 2468
 agtctaagga gcagaagtac agttttaaat ggttgccctc agaacaaaat cactgaaaga 2528
 aataaaagtt ggagactgac ctgaactcaa agcaaagaaa caaaaaaat gggaactgct 2588
 gcatttaatt aaaattaata atccttagac atgctcaaaa ggagacctca agaagtaatc 2648
 acaaaatagg acacatctag gagacagctt atttatactt aaaataaatt atattacatt 2708
 acttattaca ttgataaat gtgttggtac tattttccaa agaattatac ttttattgat 2768
 atttttgtga tatgaataaa attattttta aaaccaaaaa aaaaaaaaaa aaaaaaaaaa 2828
 aa 2830

<210> 2

<211> 304

<212> PRT

<213> Canis familiaris

<400> 2

Met Asp Tyr Thr Ala Lys Trp Arg Thr Pro Pro Leu Lys His Pro Tyr
 1 5 10 15

Leu Lys Val Ser Gln Leu Leu Val Leu Ala Ser Leu Phe Tyr Phe Cys
 20 25 30

Ser Gly Ile Ile Gln Val Asn Lys Thr Val Lys Glu Val Ala Val Leu

35	40	45	
Ser Cys Asp Tyr Asn Ile Ser Thr Thr Glu Leu Met Lys Val Arg Ile			
50	55	60	
Tyr Trp Gln Lys Asp Asp Glu Val Val Leu Ala Val Thr Ser Gly Gln			
65	70	75	80
Thr Lys Val Trp Ser Lys Tyr Glu Asn Arg Thr Phe Ala Asp Phe Thr			
85	90	95	
Asn Asn Leu Ser Ile Val Ile Met Ala Leu Arg Leu Ser Asp Asn Gly			
100	105	110	
Lys Tyr Thr Cys Ile Val Gln Lys Thr Glu Lys Arg Ser Tyr Lys Val			
115	120	125	
Lys His Met Thr Ser Val Met Leu Leu Val Arg Ala Asp Phe Pro Val			
130	135	140	
Pro Ser Ile Thr Asp Leu Gly Asn Pro Ser His Asp Ile Lys Arg Ile			
145	150	155	160
Met Cys Ser Thr Ser Gly Gly Phe Pro Lys Pro His Leu Ser Trp Trp			
165	170	175	
Glu Asn Glu Glu Glu Leu Asn Ala Ala Asn Thr Thr Val Ser Gln Asp			
180	185	190	
Pro Asp Thr Glu Leu Tyr Thr Ile Ser Ser Glu Leu Asp Phe Asn Ile			
195	200	205	
Thr Ser Asn His Ser Phe Val Cys Leu Val Lys Tyr Gly Asp Leu Thr			
210	215	220	
Val Ser Gln Ile Phe Asn Trp Gln Lys Ser Val Glu Pro His Pro Pro			
225	230	235	240
Asn Asn Gln Gln Gln Leu Trp Val Ile Leu Ile Leu Val Val Ser Gly			
245	250	255	
Val Ile Ala Val Ile Thr Ala Ile Thr Gly Gly Cys Leu Ala His Arg			
260	265	270	
Ser Ala Ala Arg Trp Arg Gln Arg Asn Arg Asn Lys Glu Asp Met Asp			
275	280	285	
Leu Glu Lys Met Ser Pro Ile Asn Ile Gly Ser Ala Gln Ala Ser Val			

290

295

300

<210> 3

<211> 2830

<212> DNA

<213> Canis familiaris

<400> 3

tttttttttt tttttttttt tttttttggt tttaaaaata atttttattca tatcacaaaa 60
 atatcaataa aagtataatt ctttggaata tagtaccaac acatttatca aatgtaataa 120
 gtaatgtaat ataatttatt ttaagtataa ataagctgtc tcttagatgt gtcctatttt 180
 gtgattactt cttgaggtct ctttttgagc atgtctaagg attattaatt ttaattaaat 240
 gcagcagttc ccattttttt tgtttctttg ctttgagtcc aggtcagttc ccaactttta 300
 tttctttcag tgattttggt ctgagggcaa ccatttaaaa ctgtacttct gtccttaga 360
 ctatatagca taatctgggc cttgatagc tgccaaaatt aaacattgta aaccagcta 420
 catgtgagaa acttgcccaa cttgtgttcc aacgtcaa atttcaa atga gaatgtctga 480
 ttcttccagt caggtctacc tccaggtcca ctgagttatg gaagacaact tcagagaaa 540
 gaatcatgag taagtgcac aggaacggca gttggccact tctttatttt taattctctg 600
 ctgatgtcag aaggatcaga aataccaagg aggaagagca acttgaggaa tctagggaaa 660
 ccctatggaa tgctattttt attacctcaa tcaaattcta aggacagcta ctgttttagta 720
 atatctgttt agtaatatat aattaaaacc tatctcattt taaatgagaa ctttctcatg 780
 ttttataaat gagttaaacc tagcataaag ccattaagga gattatcagt ccaatatgaa 840
 ccagtaggat gtgactctct aaaccaacct atccctctcc aacgtgacta tcgctttgag 900
 gtatataagt cattcatctt acatgtcagc agatattagt tttggataga tctactttct 960
 ccagaacctg atggagaagt gggaaagcaa cagaggagaa cacaatgaaa gatgagatac 1020
 tgagtaaacc ccagtaattt ttccatcatt ctcatattgg tatatatacc atcactactac 1080
 cacacattag catcagattg cttttgacag caacacaaaa ctttctagtg ttgcatcaga 1140
 tgaagtctct gaagttgacc tgacattgga catactcttt ttccagaagc attttgactg 1200

gtgtgtacac ctgtacacac cagttaatag cacagagccg gactactgat ggaaaagagc 1260
 acccgagaca ggaaatctgg gttctagctc agacctttat cattcaggtc accccctagg 1320
 ccttgatgtc cttgtcatta atgagacaag tattttccga atgtctcttc ccaggcctga 1380
 acaattcttt gaccaaggct accagagagc tacttatgtc cccaccatat tctcttggtc 1440
 actttctgca gggccactga aatgaacagg ctcttagaga acagatgcat gaagtgaag 1500
 gtgggataat cctggccatc tgaggaaatt caaagatgag gtcaatctaa gatggaggtg 1560
 ggacctccag atgttctgct catacagatg cttgggcaga tcttatgttt ataggggaca 1620
 tcttttccag gtccatgtcc tctttgttcc tatttctctg tctccatctt gcagcagatc 1680
 tgtgggctag gcagcctcct gtaatggcag tgatcacagc aatcacacca cttactacta 1740
 agatcaggat gaccagagc tgttctggt tattgggagg gtgtggctcg actgattttt 1800
 gccagttgaa gatctgtgat actgttaagt ctccatactt gacaagacac acaaagctat 1860
 ggttgcttgt tatattgaaa tccagttcac tactaatagt gtacaactca gtgtccgggt 1920
 cttgggaaac tgttgtgttg gcagcattca attcttcttc attttccac caggagaggt 1980
 gaggttttg aaaacctcca gaggttgaac acattatcct tttgatgtca tgggatggat 2040
 ttccaaggtc agttatacta gggacaggga agtcagctct gaccaataac atcacgaag 2100
 tcatgtgttt cactttgtaa gacctttttt cagtcttttg aacgatacag gtgtatttgc 2160
 cattgtctga caggcgcaga gccataatca cgatggagag gttattggtg aagtcagcaa 2220
 aggtgcgatt ctcatattg gaccacactt tcgtttgtcc agatgtgaca gccagcacca 2280
 cttcatcatc cttttgccaa tagattcgaa ctttcatcag ttctgtagtg gaaatgttgt 2340
 aatcacagga cagtactgct acttctttca ctgtcttgtt cacctggatg atgcctgaac 2400
 agaagtaaaa gagactagct agcaccaaga gctgagagac cttgagatat ggggtgttga 2460
 gtgggtgtgt tctccacttc gctgtgtaat ccatggcttc tgctgctgaa ggataactcc 2520
 tctttttttt ttttctgtg ttagaacaaa acaaaacaaa acgtcaaagg atgaggcccc 2580
 agttctgaga tgttcttttc ttgtgtctc caaaataatg gcaagttgct cagcttttct 2640

ttgggtgata taccagaggc aatccccctt ccaacttcct ttactcgct ggaagatcta 2700
 gctgatgttc attaccagca gtatttaagt caaagcaggg gatgtgtaga tggtatgtat 2760
 aacttgagga agctggttct gctgtccctt tttgtcagcc acctcagaga gtgtggctag 2820
 gacaaagcac 2830

<210> 4

<211> 912

<212> DNA

<213> Canis familiaris

<400> 4

atggattaca cagcgaagtg gagaacacca ccactcaaac acccatatct caaggtctct 60
 cagctcttgg tgctagctag tctcttttac ttctgttcag gcatcatcca ggtgaacaag 120
 acagtgaag aagtagcagt actgtcctgt gattacaaca ttccactac agaactgatg 180
 aaagttcgaa tctattggca aaaggatgat gaagtgggtc tggctgtcac atctggacaa 240
 acgaaagtgt ggtccaagta tgagaatcgc acctttgtg acttcaccaa taacctctcc 300
 atcgtgatta tggctctgcg cctgtcagac aatggcaa atacactgtat cgttcaaaag 360
 actgaaaaaa ggtcttacaa agtgaaacac atgacttcgg tgatgttatt ggtcagagct 420
 gacttcctg tccctagtat aactgacctt ggaaatccat cccatgacat caaaaggata 480
 atgtgttcaa cctctggagg ttttccaaag cctcacctct cctggtggga aaatgaagaa 540
 gaattgaatg ctgccaacac aacagtttcc caagaccgg aactgagtt gtacactatt 600
 agtagtgaac tggatttcaa tataacaagc aaccatagct ttgtgtgtct tgtcaagtat 660
 ggagacttaa cagtatcaca gatcttcaac tggcaaaaat cagtcagagcc acaccctccc 720
 aataaccagc aacagctctg ggtcatcctg atcttagtag taagtgggtg gattgctgtg 780
 atcactgcca ttacaggagg ctgcctagcc cacagatctg ctgcaagatg gagacagaga 840
 aataggaaca aagaggacat ggacctggaa aagatgtccc ctataaacat aggatctgcc 900
 caagcatctg ta 912

<210> 5
 <211> 912
 <212> DNA
 <213> Canis familiaris

<400> 5
 tacagatgct tgggcagatc ctatgtttat aggggacatc tttccaggt ccatgtcctc 60
 tttgttccta tttctctgtc tccatcttgc agcagatctg tgggctaggc agcctcctgt 120
 aatggcagtg atcacagcaa tcacaccact tactactaag atcaggatga cccagagctg 180
 ttgctgggta ttgggagggt gtggctcgac tgatttttgc cagttgaaga tctgtgatac 240
 tyttaagtct ccatacttga caagacacac aaagctatgg ttgcttgta tattgaaatc 300
 cagttcacta ctaatagtgt acaactcagt gtccgggtct tgggaaactg ttgtgttggc 360
 agcattcaat tcttcttcat tttccacca ggagagggtga ggctttggaa aacctccaga 420
 ggttgaacac attatccttt tgatgtcatg ggatggattt ccaaggtcag ttatactagg 480
 gacagggaag tcagctctga ccaataacat caccgaagtc atgtgtttca ctttgtaaga 540
 ccttttttca gtcttttgaa cgatacaggt gtatttgcca ttgtctgaca ggcgcagagc 600
 cataatcacg atggagaggt tattggtgaa gtcagcaaag gtgcgattct catacttgga 660
 ccacactttc gtttgccag atgtgacagc cagcaccact tcatcatcct tttgccaata 720
 gattcgaact ttcacaggt ctgtagtga aatggtgtaa tcacaggaca gtactgctac 780
 ttctttcact gtcttggtca cctggatgat gcctgaacag aagtaaaaga gactagctag 840
 caccaagagc tgagagacct tgagatatgg gtgtttgagt ggtggtgttc tccacttcgc 900
 tgtgtaatcc at 912

<210> 6
 <211> 1897
 <212> DNA
 <213> Canis familiaris

<220>
 <221> CDS
 <222> (6)..(992)

<400> 6

ccaag atg tat ctc aga tgc act atg gaa ctg aat aac att ctc ttt gtg 50

Met Tyr Leu Arg Cys Thr Met Glu Leu Asn Asn Ile Leu Phe Val

1

5

10

15

atg acc ctc ctg ctc tat ggt gct gct tcc atg aag agt caa gca tat 98

Met Thr Leu Leu Leu Tyr Gly Ala Ala Ser Met Lys Ser Gln Ala Tyr

20

25

30

ttc aac aag act gga gaa ctg cca tgc cat ttt aca aat tct caa aac 146

Phe Asn Lys Thr Gly Glu Leu Pro Cys His Phe Thr Asn Ser Gln Asn

35

40

45

ata agc ctg gat gag ttg gta gtg ttt tgg cag gac cag gat aag ctg 194

Ile Ser Leu Asp Glu Leu Val Val Phe Trp Gln Asp Gln Asp Lys Leu

50

55

60

gtt ctg tac gag cta tac aga ggc aaa gag aac cct caa aat gtt cat 242

Val Leu Tyr Glu Leu Tyr Arg Gly Lys Glu Asn Pro Gln Asn Val His

65

70

75

cgc aag tat aag ggc cgc aca agc ttt gac aaa gac aat tgg acc ctg 290

Arg Lys Tyr Lys Gly Arg Thr Ser Phe Asp Lys Asp Asn Trp Thr Leu

80

85

90

95

aga ctc cat aat att cag atc aag gac aag ggc ttg tat caa tgt ttc 338

Arg Leu His Asn Ile Gln Ile Lys Asp Lys Gly Leu Tyr Gln Cys Phe

100

105

110

gtt cat cat aaa ggg ccc aaa gga ctc gtt ccc atg cac cag atg aat 386

Val His His Lys Gly Pro Lys Gly Leu Val Pro Met His Gln Met Asn

115

120

125

tct gac cta tca gtg ctt gct aac ttc agt caa cct gaa ata atg gta 434

Ser Asp Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Met Val

130

135

140

act tct aat aga aca gaa aat tct ggc atc ata aat ttg acc tgc tca 482

Thr Ser Asn Arg Thr Glu Asn Ser Gly Ile Ile Asn Leu Thr Cys Ser

145

150

155

tcc ata caa ggt tac cca gaa ccc aag gag atg tat ttt ttg gta aaa 530

Ser Ile Gln Gly Tyr Pro Glu Pro Lys Glu Met Tyr Phe Leu Val Lys

160

165

170

175

acc gag aat tca agt act aag tat gat act gtc atg aag aaa tct caa 578

Thr Glu Asn Ser Ser Thr Lys Tyr Asp Thr Val Met Lys Lys Ser Gln

180 185 190

aat aat gtc aca gaa ctc tac aac gtt tct atc agc ttg tcc ttc tca 626
Asn Asn Val Thr Glu Leu Tyr Asn Val Ser Ile Ser Leu Ser Phe Ser
195 200 205

gtc cct gaa gca agc aat gtg agc atc ttc tgt gtc ctg caa ctt gag 674
Val Pro Glu Ala Ser Asn Val Ser Ile Phe Cys Val Leu Gln Leu Glu
210 215 220

tca atg aag ctt ccc tcc cta cct tat aat ata gat gca cat acg aaa 722
Ser Met Lys Leu Pro Ser Leu Pro Tyr Asn Ile Asp Ala His Thr Lys
225 230 235

ccc acc cct gat gga gac cac atc ctc tgg att gcg gct ctg ctt gta 770
Pro Thr Pro Asp Gly Asp His Ile Leu Trp Ile Ala Ala Leu Leu Val
240 245 250 255

atg ttg gtc att ttg tgt ggg atg gtg ttc ttt cta aca cta agg aaa 818
Met Leu Val Ile Leu Cys Gly Met Val Phe Phe Leu Thr Leu Arg Lys
260 265 270

agg aag aag aag cag cct ggc ccc tct cat gaa tgt gaa acc aac aaa 866
Arg Lys Lys Lys Gln Pro Gly Pro Ser His Glu Cys Glu Thr Asn Lys
275 280 285

gtg gag aga aaa gaa agt gag cag acc aag gaa aga gta cgg tac cat 914
Val Glu Arg Lys Glu Ser Glu Gln Thr Lys Glu Arg Val Arg Tyr His
290 295 300

gaa acg gaa aga tct gat gaa gcc cag tgt gtt aac att tcg aag aca 962
Glu Thr Glu Arg Ser Asp Glu Ala Gln Cys Val Asn Ile Ser Lys Thr
305 310 315

gct tca ggc gac aac agt act aca cag ttt taattaaaga gtaaagtcca 1012
Ala Ser Gly Asp Asn Ser Thr Thr Gln Phe
320 325

tccattgttt atatgccttc cctttcaaat ttggccttgc ctttttctcg tccattaata 1072

ttattattgc cactaataat aagaggcttt ccagggtcc ctctaaatga gagagcctcc 1132

ctataatgcc agttctgctc cctacaccag gagcagatgt taactgcttc tttcatctc 1192

agagcacact tgtgggcat gtcacctga ctggctcctg gtcagggaat aatgtttaag 1252

actaacacct cctgtttcag attcagcctt cttttcttaa ttttatacat tgtgttttat 1312

gtagaactcc caattactgg actaatggct tttatctatg cttaattcta agatagtgcc 1372
 tcattccatc ttgtatattt gtgactacct ctgcagctctg ggtgggagtt ttgtatgtta 1432
 tggctttata gtgttgcttt aatattttga gacataaaga gatgtgtact ataataatgt 1492
 aattactatg ccttgagaaa attctaccca ctgctgagga gctcttgctc ctctgtgagg 1552
 gtcagtagca aaatgggtggc ttggtgtgct gacaacaatg agcagaccâa ctcaaaattt 1612
 ggaagattag gaatgatgga gatagaacca gctctgagtc ctggagccac ttctatctgg 1672
 gctgctgcta atctgaggaa gatccacctg cctaacaagc tatggataag ccttagcagg 1732
 gagctctttg tgaagcagga aagcactatg cactgtgaac cctacttctc ttcttgaaaa 1792
 aaatggctga gatgatggct cagggcaact gttcaagagc caactgagag atcacaatac 1852
 ttaaaagaga aaaaagaaaa aagaaaaaaaa aaaaaaaaaa aaaaa 1897

<210> 7

<211> 329

<212> PRT

<213> Canis familiaris

<400> 7

Met Tyr Leu Arg Cys Thr Met Glu Leu Asn Asn Ile Leu Phe Val Met
 1 5 10 15

Thr Leu Leu Leu Tyr Gly Ala Ala Ser Met Lys Ser Gln Ala Tyr Phe
 20 25 30

Asn Lys Thr Gly Glu Leu Pro Cys His Phe Thr Asn Ser Gln Asn Ile
 35 40 45

Ser Leu Asp Glu Leu Val Val Phe Trp Gln Asp Gln Asp Lys Leu Val
 50 55 60

Leu Tyr Glu Leu Tyr Arg Gly Lys Glu Asn Pro Gln Asn Val His Arg
 65 70 75 80

Lys Tyr Lys Gly Arg Thr Ser Phe Asp Lys Asp Asn Trp Thr Leu Arg
 85 90 95

Leu His Asn Ile Gln Ile Lys Asp Lys Gly Leu Tyr Gln Cys Phe Val
 100 105 110

His His Lys Gly Pro Lys Gly Leu Val Pro Met His Gln Met Asn Ser
 115 120 125
 Asp Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Met Val Thr
 130 135 140
 Ser Asn Arg Thr Glu Asn Ser Gly Ile Ile Asn Leu Thr Cys Ser Ser
 145 150 155 160
 Ile Gln Gly Tyr Pro Glu Pro Lys Glu Met Tyr Phe Leu Val Lys Thr
 165 170 175
 Glu Asn Ser Ser Thr Lys Tyr Asp Thr Val Met Lys Lys Ser Gln Asn
 180 185 190
 Asn Val Thr Glu Leu Tyr Asn Val Ser Ile Ser Leu Ser Phe Ser Val
 195 200 205
 Pro Glu Ala Ser Asn Val Ser Ile Phe Cys Val Leu Gln Leu Glu Ser
 210 215 220
 Met Lys Leu Pro Ser Leu Pro Tyr Asn Ile Asp Ala His Thr Lys Pro
 225 230 235 240
 Thr Pro Asp Gly Asp His Ile Leu Trp Ile Ala Ala Leu Leu Val Met
 245 250 255
 Leu Val Ile Leu Cys Gly Met Val Phe Phe Leu Thr Leu Arg Lys Arg
 260 265 270
 Lys Lys Lys Gln Pro Gly Pro Ser His Glu Cys Glu Thr Asn Lys Val
 275 280 285
 Glu Arg Lys Glu Ser Glu Gln Thr Lys Glu Arg Val Arg Tyr His Glu
 290 295 300
 Thr Glu Arg Ser Asp Glu Ala Gln Cys Val Asn Ile Ser Lys Thr Ala
 305 310 315 320
 Ser Gly Asp Asn Ser Thr Thr Gln Phe
 325

<210> 8

<211> 1897

<212> DNA

<213> Canis familiaris

<400> 8

tttttttttt tttttttttt ttcttttttc ttttttctct tttaagtatt gtgatctctc 60
 agttggctct tgaacagttg ccttgagcca tcatctcagc catttttttc aagaagagaa 120
 gtagggttca cagtgcatag tgctttcctg cttcacaaag agctccctgc taaggcttat 180
 ccatagcttg ttaggcaggt ggatcttctc cagattagca gcagcccaga tagaagtggc 240
 tccaggactc agagctgggt ctatctccat cattoctaat cttccaaatt ttgagttggg 300
 ctgctcattg ttgtcagcac accaagccac cattttcgtc ctgacctca cagaggagca 360
 agagctctc agcagtggtt agaattttct cagggcatag taattacatt attatagtac 420
 acatctcttt atgtctcaaa atattaaagc aacactataa agccataaca tacaaaactc 480
 ccaccagac tgcagaggta gtcacaaata tacaagatgg aatgaggcac tatcttagaa 540
 ttaagcatag ataaaagcca ttagtccagt aattgggagt tctacataaa acacaatgta 600
 taaaattaag aaaagaaggc tgaatctgaa acaggagggtg ttagtcttaa acattattcc 660
 tgagccagga gccagtcagg tgagcatggc ccacaagtgt gctctgagat gaaaagaagc 720
 agttaaaatc tgctcctggt gtagggagca gaactggcat tataggaggg ctctctcatt 780
 tagagggagc cctggaaagc ctcttattat tagtggcaat aataatatta atggacgaga 840
 aaaaggcaag ccaaaatttg aaagggaagg catataaaca atggatggac tttactcttt 900
 aattaaaact gtgtagtact gttgtcgcct gaagctgtct tcgaaatggt aacacactgg 960
 gcttcacag atctttccgt ttcattgtac cgtactcttt ccttggctctg ctactttct 1020
 ttctctcca ctttgttggt ttcacattca tgagaggggc caggctgctt cttcttctt 1080
 ttccttagtg ttagaaagaa caccatccca cacaaaatga ccaacattac aagcagagcc 1140
 gcaatccaga ggatgtggtc tccatcaggg gtgggtttcg tatgtgcac tatattataa 1200
 ggtagggagg gaagcttcat tgactcaagt tgcaggacac agaagatgct cacattgctt 1260
 gcttcagggc ctgagaagga caagctgata gaaacgttgt agagttctgt gacattatct 1320
 tgagatttct tcatgacagt atcatactta gtacttgaat tctcggtttt taccaaaaaa 1380
 tacatctctt tgggttctgg gtaaccttgt atggatgagc aggtcaaatt tatgatgcca 1440

gaattttctg ttctattaga agttaccatt atttcaggtt gactgaagtt agcaagcact 1500
 gataggtcag aattcatctg gtgcatggga acgagtcctt tgggcccttt atgatgaacg 1560
 aaacattgat acaagccctt gtccttgatc tgaatattat ggagtctcag ggtccaattg 1620
 tctttgtcaa agcttgtgcg gcccttatac ttgcatgaa cattttgagg gttctctttg 1680
 cctctgtata gctcgtacag aaccagctta tccctggctc gccaaaacac taccaactca 1740
 tccaggctta tgttttgaga atttgtaaaa tggcatggca gttctccagt cttgttgaaa 1800
 tatgcttgac tcttcattga agcagcacca tagagcagga gggatcatcac aaagagaatg 1860
 ttattcagtt ccatagtgc tctgagatac atcttgg 1897

<210> 9

<211> 987

<212> DNA

<213> Canis familiaris

<400> 9

atgtatctca gatgcactat ggaactgaat aacattctct ttgtgatgac cctcctgctc 60
 tatggtgctg cttccatgaa gagtcaagca tatttcaaca agactggaga actgccatgc 120
 cattttacaa attctcaaaa cataagcctg gatgagttgg tagtgttttg gcaggaccag 180
 gataagctgg ttctgtacga gctatacaga ggcaaagaga accctcaaaa tgttcacgc 240
 aagtataagg gccgcacaag ctttgacaaa gacaattgga cctgagact ccataatatt 300
 cagatcaagg acaagggctt gtatcaatgt ttctgtcatc ataaagggcc caaaggactc 360
 gttcccatgc accagatgaa ttctgacctt tcagtgttg ctaacttcag tcaacctgaa 420
 ataatggtaa cttctaatag aacagaaaat tctggcatca taaatttgac ctgctcatcc 480
 atacaaggtt acccagaacc caaggagatg tattttttgg taaaaaccga gaattcaagt 540
 actaagtatg atactgtcat gaagaaatct caaaataatg tcacagaact ctacaacgtt 600
 tctatcagct tgtccttctc agtccttgaa gcaagcaatg tgagcatctt ctgtgtcctg 660
 caacttgagt caatgaagct tccctcccta cttataata tagatgcaca tacgaaaccc 720

acccctgatg gagaccacat cctctggatt gcggtctctgc ttgtaatgtt ggtcattttg 780
 tgtgggatgg tgttctttct aacactaagg aaaaggaaga agaagcagcc tggccctct 840
 catgaatgtg aaaccaacaa agtggagaga aaagaaagtg agcagaccaa ggaaagagta 900
 cgggtaccatg aaacggaaag atctgatgaa gcccagtgtg ttaacatttc gaagacagct 960
 tcaggcgaca acagtactac acagttt 987

<210> 10

<211> 987

<212> DNA

<213> Canis familiaris

<400> 10

aaactgtgta gtactgttgt cgctgaagc tgtcttcgaa atgttaacac actgggcttc 60
 atcagatctt tccgtttcat ggtaccgtac tctttccttg gtctgtctac tttcttttct 120
 ctccactttg ttggtttcac attcatgaga ggggccaggc tgcttcttct tctttttcct 180
 tagtgttaga aagaacacca tccacacaaa aatgaccaac attacaagca gagccgcaat 240
 ccagaggatg tggctctcat caggggtggg ttctgtatgt gcctctatat tataaggtag 300
 ggaggggaagc ttcattgact caagttgcag gacacagaag atgtctacat tgcttgcttc 360
 agggactgag aaggacaagc tgatagaaac gttgtagagt tctgtgacat tattttgaga 420
 tttcttcatg acagtatcat acttagtact tgaattctcg gtttttacca aaaaatacat 480
 ctcttgggt tctgggtaac cttgtatgga tgagcaggtc aaatttatga tgccagaatt 540
 ttctgttcta ttagaagtta ccattatttc aggttgactg aagttagcaa gcactgatag 600
 gtcagaattc atctgggtgca tgggaacgag tctttgggc cttttatgat gaacgaaaca 660
 ttgatacaag cccttgctct tgatctgaat attatggagt ctcagggtcc aattgtcttt 720
 gtcaaagctt gtgcggccct tatacttgcg atgaacattt tgagggttct ctttgctct 780
 gtatagctcg tacagaacca gcttatcctg gtctgcca aacactacca actcatccag 840
 gcttatgttt tgagaatttg taaaatggca tggcagttct ccagtcttgt tgaaatatgc 900
 ttgactcttc atggaagcag caccatagag caggagggtc atcacaaga gaatgttatt 960

cagttccata gtgcattctga gatacat

987

<210> 11

<211> 1024

<212> DNA

<213> Canis familiaris

<220>

<221> CDS

<222> (79)..(783)

<400> 11

ctttgacggtt ttgttttgggt ttgttctaac acaagaaaaa aaaaaaagag gagttatcct 60

tcagcagcag	cagaagcc	atg	gat	tac	aca	gcg	aag	tgg	aga	aca	cca	cca	111
		Met	Asp	Tyr	Thr	Ala	Lys	Trp	Arg	Thr	Pro	Pro	
		1				5					10		

ctc	aaa	cac	cca	tat	ctc	aag	gtc	tct	cag	ctc	ttg	gtg	cta	gct	agt	159
Leu	Lys	His	Pro	Tyr	Leu	Lys	Val	Ser	Gln	Leu	Leu	Val	Leu	Ala	Ser	
		15					20						25			

ctc	ttt	tac	ttc	tgt	tca	ggc	atc	atc	cag	gtg	aac	aag	aca	gtg	aaa	207
Leu	Phe	Tyr	Phe	Cys	Ser	Gly	Ile	Ile	Gln	Val	Asn	Lys	Thr	Val	Lys	
		30					35					40				

gaa	gta	gca	gta	ctg	tcc	tgt	gat	tac	aac	att	tcc	act	aca	gaa	ctg	255
Glu	Val	Ala	Val	Leu	Ser	Cys	Asp	Tyr	Asn	Ile	Ser	Thr	Thr	Glu	Leu	
		45					50					55				

atg	aaa	gtt	cga	atc	tat	tgg	caa	aag	gat	gat	gaa	gtg	gtg	ctg	gct	303
Met	Lys	Val	Arg	Ile	Tyr	Trp	Gln	Lys	Asp	Asp	Glu	Val	Val	Leu	Ala	
	60					65					70			75		

gtc	aca	tct	gga	caa	acg	aaa	gtg	tgg	tcc	aag	tat	gag	aat	cgc	acc	351
Val	Thr	Ser	Gly	Gln	Thr	Lys	Val	Trp	Ser	Lys	Tyr	Glu	Asn	Arg	Thr	
			80					85					90			

ttt	gct	gac	ttc	acc	aat	aac	ctc	tcc	atc	gtg	att	atg	gct	ctg	cgc	399
Phe	Ala	Asp	Phe	Thr	Asn	Asn	Leu	Ser	Ile	Val	Ile	Met	Ala	Leu	Arg	
			95					100					105			

ctg	tca	gac	aat	ggc	aaa	tac	acc	tgt	atc	gtt	caa	aag	act	gaa	aaa	447
Leu	Ser	Asp	Asn	Gly	Lys	Tyr	Thr	Cys	Ile	Val	Gln	Lys	Thr	Glu	Lys	
		110					115					120				

agg tct tac aaa gtg aaa cac atg act tcg gtg atg tta ttg gtc aga 495
 Arg Ser Tyr Lys Val Lys His Met Thr Ser Val Met Leu Leu Val Arg
 125 130 135

gct gac ttc cct gtc cct agt ata act gac ctt gga aat cca tcc cat 543
 Ala Asp Phe Pro Val Pro Ser Ile Thr Asp Leu Gly Asn Pro Ser His
 140 145 150 155

gac atc aaa agg ata atg tgt tca acc tct gga ggt ttt cca aag cct 591
 Asp Ile Lys Arg Ile Met Cys Ser Thr Ser Gly Gly Phe Pro Lys Pro
 160 165 170

cac ctc tcc tgg tgg gaa aat gaa gaa gaa ttg aat gct gcc aac aca 639
 His Leu Ser Trp Trp Glu Asn Glu Glu Glu Leu Asn Ala Ala Asn Thr
 175 180 185

aca gtt tcc caa gac ccg gac act gag ttg tac act att agt agt gaa 687
 Thr Val Ser Gln Asp Pro Asp Thr Glu Leu Tyr Thr Ile Ser Ser Glu
 190 195 200

ctg gat ttc aat ata aca agc aac cat agc ttt gtg tgt ctt gtc aag 735
 Leu Asp Phe Asn Ile Thr Ser Asn His Ser Phe Val Cys Leu Val Lys
 205 210 215

tat gga gac tta aca gta tca cag atc ttc aac tgg caa aaa tgt aag 783
 Tyr Gly Asp Leu Thr Val Ser Gln Ile Phe Asn Trp Gln Lys Cys Lys
 220 225 230 235

taacattggt ctgaggagtt tctactgtgt aaaatctaaa aagaaaataa ctcagccaga 843

tacatttttg aattatgtat gttaactttg atagcatttc ttgtattttt agaccataa 903

atgataatga agtgatattg tgacttggtta aggtcactgt acaggtatgg ccataatggt 963

actaatttta tttcctttaa taaaccttct aaaactgaga catccaaaaa aaaaaaaaaa 1023

a 1024

<210> 12

<211> 235

<212> PRT

<213> Canis familiaris

<400> 12

Met Asp Tyr Thr Ala Lys Trp Arg Thr Pro Pro Leu Lys His Pro Tyr
 1 5 10 15

Leu Lys Val Ser Gln Leu Leu Val Leu Ala Ser Leu Phe Tyr Phe Cys
20 25 30

Ser Gly Ile Ile Gln Val Asn Lys Thr Val Lys Glu Val Ala Val Leu
35 40 45

Ser Cys Asp Tyr Asn Ile Ser Thr Thr Glu Leu Met Lys Val Arg Ile
50 55 60

Tyr Trp Gln Lys Asp Asp Glu Val Val Leu Ala Val Thr Ser Gly Gln
65 70 75 80

Thr Lys Val Trp Ser Lys Tyr Glu Asn Arg Thr Phe Ala Asp Phe Thr
85 90 95

Asn Asn Leu Ser Ile Val Ile Met Ala Leu Arg Leu Ser Asp Asn Gly
100 105 110

Lys Tyr Thr Cys Ile Val Gln Lys Thr Glu Lys Arg Ser Tyr Lys Val
115 120 125

Lys His Met Thr Ser Val Met Leu Leu Val Arg Ala Asp Phe Pro Val
130 135 140

Pro Ser Ile Thr Asp Leu Gly Asn Pro Ser His Asp Ile Lys Arg Ile
145 150 155 160

Met Cys Ser Thr Ser Gly Gly Phe Pro Lys Pro His Leu Ser Trp Trp
165 170 175

Glu Asn Glu Glu Glu Leu Asn Ala Ala Asn Thr Thr Val Ser Gln Asp
180 185 190

Pro Asp Thr Glu Leu Tyr Thr Ile Ser Ser Glu Leu Asp Phe Asn Ile
195 200 205

Thr Ser Asn His Ser Phe Val Cys Leu Val Lys Tyr Gly Asp Leu Thr
210 215 220

Val Ser Gln Ile Phe Asn Trp Gln Lys Cys Lys
225 230 235

<210> 13

<211> 1024

<212> DNA

<213> Canis familiaris

<400> 13

tttttttttt ttttttgat gtctcagttt tagaaggttt attaaaggaa ataaaattag 60
 taacattatg gccatacctg tacagtgacc ttaacaagtc acaatatcac ttcattatca 120
 tttatgggtc taaaaataca agaaatgcta tcaaagttaa catacataat tccaaaatgt 180
 atctggctga gttattttct ttttagattt tacacagtag aaactcctca gaacaatgtt 240
 acttacattt ttgccagttg aagatctgtg atactgttaa gtctccatac ttgacaagac 300
 acacaaagct atggttgctt gttatattga aatccagttc actactaata gtgtacaact 360
 cagtgtccgg gtcttgggaa actgttgtgt tggcagcatt caattcttct tcattttccc 420
 accaggagag gtgaggcttt ggaaaacctc cagaggttga acacattatc cttttgatgt 480
 catgggatgg atttccaagg tcagttatac tagggacagg gaagtcagct ctgaccaata 540
 acatcaccga agtcatgtgt ttcactttgt aagacctttt ttcagtcttt tgaacgatac 600
 aggtgtattt gccattgtct gacaggcgca gagccataat cacgatggag aggttattgg 660
 tgaagtcagc aaaggtgcga ttctcatact tggaccacac ttctgtttgt ccagatgtga 720
 cagccagcac cacttcatca tccttttgcc aatagattcg aactttcatc agttctgtag 780
 tggaaatggt gtaatcacag gacagtactg ctacttcttt cactgtcttg ttcacctgga 840
 tgatgcctga acagaagtaa aagagactag ctagcaccaa gagctgagag accttgagat 900
 atgggtgttt gagtgggtgt gttctccact tcgctgtgta atccatggct tctgctgctg 960
 ctgaaggata actcctcttt ttttttttct ttgtgttaga acaaaacaaa acaaaacgtc 1020
 aaag 1024

<210> 14

<211> 705

<212> DNA

<213> Canis familiaris

<400> 14

atggattaca cagcgaagtg gagaacacca cactcaaac acccatatct caaggtctct 60
 cagctcttgg tgctagctag tctcttttac ttctgttcag gcatcatcca ggtgaacaag 120

acagtgaag aagtagcagt actgtcctgt gattacaaca ttccactac agaactgatg 180
 aaagtgcgaa tctattggca aaaggatgat gaagtgggac tggctgtcac atctggacaa 240
 acgaaagtgt ggtccaagta tgagaatcgc accttgctg acttcaccaa taacctctcc 300
 atcgtgatta tggctctgcg cctctcagac aatggcaa atacctgtat cgttcaaaag 360
 actgaaaaaa ggtcttaca agtgaaacac atgacttcgg tgatgttatt ggtcagagct 420
 gacttcctg tccctagtat aactgacctt ggaaatccat cccatgacat caaaaggata 480
 atgtgttcaa cctctggagg tttccaaaag cctcacctct cctgggtgga aatgaagaa 540
 gaattgaatg ctgccaacac aacagtttcc caagaccgg aactgagtt gtacactatt 600
 agtagtgaac tggatttcaa tataacaagc aaccatagct ttgtgtgtct tgtcaagtat 660
 ggagacttaa cagtatcaca gatcttcaac tggcaaaaat gtaag 705

<210> 15

<211> 705

<212> DNA

<213> Canis familiaris

<400> 15

cttacatttt tgccagttga agatctgtga tactgttaag tctccatact tgacaagaca 60
 caciaagcta tggttgcttg ttatattgaa atccagttca ctactaatag tgtacaactc 120
 agtgtccggg tcttgggaaa ctgttgtgtt ggcagcattc aattottttt cattttocca 180
 ccaggagagg tgaggctttg gaaaacctcc agaggttgaa cacattatcc tttgatgtc 240
 atgggatgga tttccaaggt cagttatact agggacaggg aagtcagctc tgaccaataa 300
 catcacgaa gtcattgtgtt tcaatttgta agacctttt tcagctttt gaacgatata 360
 ggtgtatttg ccattgtctg acaggcgcag agccataatc acgatggaga ggttattggt 420
 gaagtcagca aaggtgcgat tctcatactt ggaccacact ttcgtttgtc cagatgtgac 480
 agccagcacc acttcatcat ccttttgcca atagattoga actttcatca gttctgtagt 540
 ggaaatgttg taatcacagg acagtactgc tacttcttcc actgtcttgt tcacctggat 600
 gatgcctgaa cagaagtaaa agagactagc tagcaccaag agctgagaga ccttgagata 660

tggggtgtttg agtggtggtg ttctccactt cgctgtgtaa tccat

705

<210> 16

<211> 1795

<212> DNA

<213> Canis familiaris

<220>

<221> CDS

<222> (7)..(846)

<400> 16

gccaaag atg tat ctc aga tgc act atg gaa ctg aat aac att ctc ttt 48
Met Tyr Leu Arg Cys Thr Met Glu Leu Asn Asn Ile Leu Phe
1 5 10

gtg atg acc ctc ctg ctc tat ggt gct gct tcc atg aag agt caa gca 96
Val Met Thr Leu Leu Leu Tyr Gly Ala Ala Ser Met Lys Ser Gln Ala
15 20 25 30

tat ttc aac aag act gga gaa ctg cca tgc cat ttt aca aat tct caa 144
Tyr Phe Asn Lys Thr Gly Glu Leu Pro Cys His Phe Thr Asn Ser Gln
35 40 45

aac ata agc ctg gat gag ttg gta gtg ttt tgg cag gac cag gat aag 192
Asn Ile Ser Leu Asp Glu Leu Val Val Phe Trp Gln Asp Gln Asp Lys
50 55 60

ctg gtt ctg tac gag cta tac aga ggc aaa gag aac cct caa aat gtt 240
Leu Val Leu Tyr Glu Leu Tyr Arg Gly Lys Glu Asn Pro Gln Asn Val
65 70 75

cat cgc aag tat aag ggc cgc aca agc ttt gac aaa gac aat tgg acc 288
His Arg Lys Tyr Lys Gly Arg Thr Ser Phe Asp Lys Asp Asn Trp Thr
80 85 90

ctg aga ctc cat aat att cag atc aag gac aag ggc ttg tat caa tgt 336
Leu Arg Leu His Asn Ile Gln Ile Lys Asp Lys Gly Leu Tyr Gln Cys
95 100 105 110

ttc gtt cat cat aaa ggg ccc aaa gga ctc gtt ccc atg cac cag atg 384
Phe Val His His Lys Gly Pro Lys Gly Leu Val Pro Met His Gln Met
115 120 125

aat tct gac cta tca gtg ctt gct aac ttc agt caa cct gaa ata atg 432
Asn Ser Asp Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Met

130 135 140
 gta act tct aat aga aca gaa aat tct ggc atc ata aat ttg acc tgc 480
 Val Thr Ser Asn Arg Thr Glu Asn Ser Gly Ile Ile Asn Leu Thr Cys
 145 150 155
 tca tcc ata caa ggt tac cca gaa ccc aag gag atg tat ttt ttg gta 528
 Ser Ser Ile Gln Gly Tyr Pro Glu Pro Lys Glu Met Tyr Phe Leu Val
 160 165 170
 aaa acc gag aat tca agt act aag tat gat act gtc atg aag aaa tct 576
 Lys Thr Glu Asn Ser Ser Thr Lys Tyr Asp Thr Val Met Lys Lys Ser
 175 180 185 190
 caa aat aat gtc aca gaa ctc tac aac gtt tct atc agc ttg tcc ttc 624
 Gln Asn Asn Val Thr Glu Leu Tyr Asn Val Ser Ile Ser Leu Ser Phe
 195 200 205
 tca gtc cct gaa gca agc aat gtg agc atc ttc tgt gtc ctg caa ctt 672
 Ser Val Pro Glu Ala Ser Asn Val Ser Ile Phe Cys Val Leu Gln Leu
 210 215 220
 gag tca atg aag ctt ccc tcc cta cct tat aat ata gaa acc aac aaa 720
 Glu Ser Met Lys Leu Pro Ser Leu Pro Tyr Asn Ile Glu Thr Asn Lys
 225 230 235
 gtg gag aga aaa gaa agt gag cag acc aag gaa aga gta cgg tac cat 768
 Val Glu Arg Lys Glu Ser Glu Gln Thr Lys Glu Arg Val Arg Tyr His
 240 245 250
 gaa acg gaa aga tct gat gaa gcc cag tgt gtt aac att tcg aag aca 816
 Glu Thr Glu Arg Ser Asp Glu Ala Gln Cys Val Asn Ile Ser Lys Thr
 255 260 265 270
 gct tca ggc gac aac agt act aca cag ttt taattaaaga gtaaagtcca 866
 Ala Ser Gly Asp Asn Ser Thr Thr Gln Phe
 275 280
 tccattgttt atatgccttc cctttcaaatt ttgggttgc ctttttctcg tccattaata 926
 ttattattgc cactaataat aagaggcttt ccagggtcc ctctaaatga gagagcctcc 986
 ctataatgcc agttctgctc cctacaccag gacagattt taactgcttc ttttcatctc 1046
 agagcacact tgtgggccat gctcacctga ctggctctg gctcaggaat aatgtttaag 1106
 actaacacct cctgtttcag attcagcctt cttttcttaa ttttatacat tgtgttttat 1166

gtagaactcc caattactgg actaatggct tttatctatg ctttaattcta agatagtgcc 1226
 tcattccatc ttgtatatatt gtgactacct ctgcagtcctg ggtgggagtt ttgtatgtta 1286
 tggctttata gtgttgcttt aatattttga gacataaaga gatgtgtact ataataatgt 1346
 aattactatg ccctgagaaa attctaccca ctgctgagga gctcttgctc ctctgtgagg 1406
 gtcagtagca aaatgggtggc ttggtgtgct gacaacaatg agcagaccaa ctcaaaattt 1466
 ggaagattag gaatgatgga gatagaacca gctctgagtc ctggagccac ttctatctgg 1526
 gctgctgcta atctgaggaa gatccacctg cctaacaagc tatggataag ccttagcagg 1586
 gagctctttg tgaagcagga aagcactatg cactgtgaac cctacttctc ttcttgaaaa 1646
 aaatggctga gatgatggct cagggcaact gttcaagagc caactgagag atcacaatac 1706
 ttaaaagaga aaaaagaaaa aagaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1766
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1795

<210> 17

<211> 280

<212> PRT

<213> Canis familiaris

<400> 17

Met Tyr Leu Arg Cys Thr Met Glu Leu Asn Asn Ile Leu Phe Val Met
 1 5 10 15

Thr Leu Leu Leu Tyr Gly Ala Ala Ser Met Lys Ser Gln Ala Tyr Phe
 20 25 30

Asn Lys Thr Gly Glu Leu Pro Cys His Phe Thr Asn Ser Gln Asn Ile
 35 40 45

Ser Leu Asp Glu Leu Val Val Phe Trp Gln Asp Gln Asp Lys Leu Val
 50 55 60

Leu Tyr Glu Leu Tyr Arg Gly Lys Glu Asn Pro Gln Asn Val His Arg
 65 70 75 80

Lys Tyr Lys Gly Arg Thr Ser Phe Asp Lys Asp Asn Trp Thr Leu Arg
 85 90 95

Leu His Asn Ile Gln Ile Lys Asp Lys Gly Leu Tyr Gln Cys Phe Val

100 105 110
 His His Lys Gly Pro Lys Gly Leu Val Pro Met His Gln Met Asn Ser
 115 120 125
 Asp Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Met Val Thr
 130 135 140
 Ser Asn Arg Thr Glu Asn Ser Gly Ile Ile Asn Leu Thr Cys Ser Ser
 145 150 155 160
 Ile Gln Gly Tyr Pro Glu Pro Lys Glu Met Tyr Phe Leu Val Lys Thr
 165 170 175
 Glu Asn Ser Ser Thr Lys Tyr Asp Thr Val Met Lys Lys Ser Gln Asn
 180 185 190
 Asn Val Thr Glu Leu Tyr Asn Val Ser Ile Ser Leu Ser Phe Ser Val
 195 200 205
 Pro Glu Ala Ser Asn Val Ser Ile Phe Cys Val Leu Gln Leu Glu Ser
 210 215 220
 Met Lys Leu Pro Ser Leu Pro Tyr Asn Ile Glu Thr Asn Lys Val Glu
 225 230 235 240
 Arg Lys Glu Ser Glu Gln Thr Lys Glu Arg Val Arg Tyr His Glu Thr
 245 250 255
 Glu Arg Ser Asp Glu Ala Gln Cys Val Asn Ile Ser Lys Thr Ala Ser
 260 265 270
 Gly Asp Asn Ser Thr Thr Gln Phe
 275 280

<210> 18

<211> 1795

<212> DNA

<213> Canis familiaris

<400> 18

tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 60

tttttttttt tttttttttt ctcttttaag tattgtgata tctcagttgg ctcttgaaca 120

gttgccctga gccatcatct cagccatttt ttccaagaag agaagtaggg ttcacagtgc 180

atagtgcctt cctgcttcac aaagagctcc ctgctaaggc ttatccatag cttgttaggc 240
 aggtggatct tcctcagatt agcagcagcc cagatagaag tggctccagg actcagagct 300
 ggttctatct ccatcattcc taatcttcca aattttgagt tggctctgctc attgttgtca 360
 gcacaccaag ccaccatttt cgtactgacc ctcacagagg agcaagagct cctcagcagt 420
 gggtagaatt ttctcagggc atagtaatta cattattata gtacacatct ctttatgtct 480
 caaaatatta aagcaacact ataaagccat aacatacaaa actcccaccc agactgcaga 540
 ggtagtcaca aatatacaag atggaatgag gcactatctt agaattaago atagataaaa 600
 gccattagtc cagtaattgg gagttctaca taaaacacaa tgtataaaat taagaaaaga 660
 aggctgaatc tgaaacagga ggtgttagtc ttaaacatta ttctgagcc aggagccagt 720
 caggtgagca tggcccacaa gtgtgctctg agatgaaaag aagcagttaa aatctgctcc 780
 tgggtgtaggg agcagaactg gcattatagg gaggtctctc catttagagg gagccctgga 840
 aagcctctta ttattagtgg caataataat attaattggac gagaaaaagg caagccaaaa 900
 tttgaaaggg aaggcatata aacaatggat ggactttact ctttaattaa aactgtgtag 960
 tactgttgct gcctgaagct gtcttcgaaa tgtaacaca ctgggcttca tcagatcttt 1020
 ccgtttcatg gtaccgtact ctttccttgg tctgctcact ttcttttctc tccactttgt 1080
 tggtttctat attataaggt agggagggaa gtttcattga ctcaagttgc aggacacaga 1140
 agatgctcac attgcttgct tcagggaactg agaaggacaa gctgatagaa acgttgtaga 1200
 gttctgtgac attatattga gattttctca tgacagtatc atacttagta cttgaattct 1260
 cggtttttac caaaaaatac atctccttgg gttctgggta acctgtatg gatgagcagg 1320
 tcaaatattat gatgccagaa tttctgttcc tattagaagt taccattatt tcaggttgac 1380
 tgaagttagc aagcactgat aggtcagaat tcctctgggtg catgggaacg agtcctttgg 1440
 gccctttatg atgaacgaaa cattgataca agcccttgct cttgatctga atattatgga 1500
 gtctcagggc ccaattgtct ttgtcaaagc ttgtgcggcc cttatacttg cgatgaacat 1560
 tttgaggggt ctctttgcct ctgtatagct cgtacagaac cagcttatcc tggctcctgcc 1620

aaaacactac caactcatcc aggcttatgt ttgagaatt tgtaaaatgg catggcagtt 1680
 ctccagtctt gttgaaatat gcttgactct tcatggaagc agcaccatag agcaggagg 1740
 tcatcacaaa gagaatgtta ttcagttcca tagtgcatct gagatacatc ttggc 1795

<210> 19

<211> 840

<212> DNA

<213> Canis familiaris

<400> 19

atgtatctca gatgcactat ggaactgaat aacattctct ttgtgatgac cctcctgctc 60
 tatggtgctg ctcccatgaa gagtcaagca tatttcaaca agactggaga actgccatgc 120
 cattttacaa attctcaaaa cataagcctg gatgagttgg tagtgttttg gcaggaccag 180
 gataagctgg ttctgtacga gctatacaga ggcaaagaga accctcaaaa tgttcatcgc 240
 aagtataagg gccgcacaag ctttgacaaa gacaattgga ccttgagact ccataatatt 300
 cagatcaagg acaagggctt gtatcaatgt ttcgttcac ataaagggcc caaaggactc 360
 gttcccatgc accagatgaa ttctgacctc tcagtgttg ctaacttcag tcaacctgaa 420
 ataatggtaa cttctaatag aacagaaaat tctggcatca taaatttgac ctgctcatcc 480
 atacaagggt acccagaacc caaggagatg tattttttgg taaaaaccga gaattcaagt 540
 actaagtatg atactgtcat gaagaaatct caaaataatg tcacagaact ctacaacgtt 600
 tctatcagct tgtccttctc agtccttgaa gcaagcaatg tgagcatctt ctgtgtcctg 660
 caacttgagt caatgaagct tccctcccta ccttataata tagaaaccaa caaagtggag 720
 agaaaagaaa gtgagcagac caaggaaaga gtacgttacc atgaaacgga aagatctgat 780
 gaagcccagt gtgttaacat ttcgaagaca gcttcaggcg acaacagtac tacacagttt 840

<210> 20

<211> 840

<212> DNA

<213> Canis familiaris

<400> 20

aaactgtgta gtactgttgt cgctgaagc tgtcttcgaa atgttaacac actgggcttc 60
 atcagatctt tccgtttcat ggtaccgtac ttttccttg gtctgtcac tttcttttct 120
 ctccactttg ttggtttcta tattataagg tagggaggga agcttcattg actcaagttg 180
 caggacacag aagatgctca cattgcttgc ttcagggact gagaaggaca agctgataga 240
 aacgttgtag agttctgtga cattatcttg agatttcttc atgacagtat catacttagt 300
 acttgaattc tcggttttta ccaaaaaata catctccttg ggttctgggt aacottgtat 360
 ggatgagcag gtcaaattta tgatgccaga attttctgtt ctattagaag ttaccattat 420
 ttcaggttga ctgaagttag caagcactga taggtcagaa ttcattctgtt gcatgggaac 480
 gagtcctttg ggccctttat gatgaacgaa acattgatac aagcccttgt ccttgatctg 540
 aatattatgg agtctcaggg tccaattgtc tttgtcaaag cttgtgcggc cttataactt 600
 gcgatgaaca ttttgagggt tctctttgcc tctgtatagc tegtacagaa ccagcttatt 660
 ctggtcctgc caaaacacta ccaactcatc caggcttatg ttttgagaat ttgtaaaatg 720
 gcatggcagt tctccagtct tgttgaaata tgcttgactc ttcattggaag cagcaccata 780
 gagcaggagg gtcattcaca agagaatgtt attcagttcc atagtgcac tgagatacat 840

<210> 21
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic
 Primer

<400> 21
 gtcaraagctg acttcctt 18

<210> 22
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 22

gtagaaactc ctcagaacaa tg

22

<210> 23

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 23

gtagtatattt ggcaggacc

19

<210> 24

<211> 23

<212> DNA

<213> Canis familiaris

<400> 24

tagaygsgca ggtcaaattt atg

23

<210> 25

<211> 2830

<212> DNA

<213> Felis catus

<220>

<221> CDS

<222> (179)..(1174)

<400> 25

gttttttttt ttttgagttc tagtctcagc cctgacatta tttctttctc tacaaagagt 60

gttaggaagt tatggggagc tcacaaaggc tctcatcgt ttattcttaa caccttgttt 120

ctgtgttcct cggaatgtc actgagctta tacatctggt ctctgggagc tgcagtgg 178

atg ggc att tgt gac agc act atg gga ctg agt cac act ctc ctt gtg 226

Met Gly Ile Cys Asp Ser Thr Met Gly Leu Ser His Thr Leu Leu Val

1

5

10

15

atg gcc ctc ctg ctc tct ggt gtt tct tcc atg aag agt caa gca tat 274
 Met Ala Leu Leu Leu Ser Gly Val Ser Ser Met Lys Ser Gln Ala Tyr
 20 25 30

ttc aac aag act gga gaa ctg cca tgc cat ttt aca aac tct caa aac 322
 Phe Asn Lys Thr Gly Glu Leu Pro Cys His Phe Thr Asn Ser Gln Asn
 35 40 45

ata agc ctg gat gag ctg gta gta ttt tgg cag gac cag gat aag ctg 370
 Ile Ser Leu Asp Glu Leu Val Val Phe Trp Gln Asp Gln Asp Lys Leu
 50 55 60

gtt ctg tat gag ata ttc aga ggc aaa gag aac cct caa aat gtt cat 418
 Val Leu Tyr Glu Ile Phe Arg Gly Lys Glu Asn Pro Gln Asn Val His
 65 70 75 80

ctc aaa tat aag ggc cgt aca agc ttt gac aag gac aac tgg acc ctg 466
 Leu Lys Tyr Lys Gly Arg Thr Ser Phe Asp Lys Asp Asn Trp Thr Leu
 85 90 95

aga ctc cac aat gtt cag atc aag gac aag ggc aca tat cac tgt ttc 514
 Arg Leu His Asn Val Gln Ile Lys Asp Lys Gly Thr Tyr His Cys Phe
 100 105 110

att cat tat aaa ggg ccc aaa gga cta gtt ccc atg cac caa atg agt 562
 Ile His Tyr Lys Gly Pro Lys Gly Leu Val Pro Met His Gln Met Ser
 115 120 125

tct gac cta tca gtg ctt gct aac ttc agt caa cct gaa ata aca gta 610
 Ser Asp Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Thr Val
 130 135 140

act tct aat aga aca gaa aat tct ggc atc ata aat ttg acc tgc tca 658
 Thr Ser Asn Arg Thr Glu Asn Ser Gly Ile Ile Asn Leu Thr Cys Ser
 145 150 155 160

tct ata caa ggt tac cca gaa cct aag gag atg tat ttt cag cta aac 706
 Ser Ile Gln Gly Tyr Pro Glu Pro Lys Glu Met Tyr Phe Gln Leu Asn
 165 170 175

act gag aat tca act act aag tat gat act gtc atg aag aaa tct caa 754
 Thr Glu Asn Ser Thr Thr Lys Tyr Asp Thr Val Met Lys Lys Ser Gln
 180 185 190

aat aat gtg aca gaa ctg tac aac gtt tct atc agc ttg cct ttt tca 802
 Asn Asn Val Thr Glu Leu Tyr Asn Val Ser Ile Ser Leu Pro Phe Ser
 195 200 205

gtc cct gaa gca cac aat gtg agc gtc ttt tgt gcc ctg aaa ctg gag 850
 Val Pro Glu Ala His Asn Val Ser Val Phe Cys Ala Leu Lys Leu Glu
 210 215 220

aca ctg gag atg ctg ctc tcc cta cct ttc aat ata gat gca caa cct 898
 Thr Leu Glu Met Leu Leu Ser Leu Pro Phe Asn Ile Asp Ala Gln Pro
 225 230 235 240

aag gat aaa gac cct gaa caa ggc cac ttc ctc tgg att gcg gct gta 946
 Lys Asp Lys Asp Pro Glu Gln Gly His Phe Leu Trp Ile Ala Ala Val
 245 250 255

ctt gta atg ttt gtt gtt ttt tgt ggg atg gtg tcc ttt aaa aca cta 994
 Leu Val Met Phe Val Val Phe Cys Gly Met Val Ser Phe Lys Thr Leu
 260 265 270

agg aaa agg aag aag aag cag cct ggc ccc tct cat gaa tgt gaa acc 1042
 Arg Lys Arg Lys Lys Lys Gln Pro Gly Pro Ser His Glu Cys Glu Thr
 275 280 285

atc aaa agg gag aga aaa gag agc aaa cag acc aac gaa aga gta cca 1090
 Ile Lys Arg Glu Arg Lys Glu Ser Lys Gln Thr Asn Glu Arg Val Pro
 290 295 300

tac cac gta cct gag aga tct gat gaa gcc cag tgt att aac att ttg 1138
 Tyr His Val Pro Glu Arg Ser Asp Glu Ala Gln Cys Ile Asn Ile Leu
 305 310 315 320

aag aca gcc tca ggc gac aaa agt act aca cat ttt taattaaaga 1184
 Lys Thr Ala Ser Gly Asp Lys Ser Thr Thr His Phe
 325 330

ataaagtcca tataactgtc cattgtttat atgcctttcc cttcaagttt tgggcttacc 1244
 tttttttgtc tattaatatt attattacca ttaataatag tggagggttcc aggactccat 1304
 ctgagaaagc caccctgtaa tgccagctct gtcctctacc tcaggagcag accttaactg 1364
 cttcttttca tttcagagca aatttgtgag ccaagctcac ctgactggat cctggctcag 1424
 gaataatggt taagactaac acctcctggt tcacattcag cttcttttcc ttaattttat 1484
 aaattgcgtc ttatgtagaa ctcccaatta ctggaataat ggctttttatc tatgtaattc 1544
 taaggtagtg cctcattcta tcttgtatat ttgtgactga ataactacct cttcagttct 1604
 gtgggagtta tatattttat ggctttttata gtattgctat taatatcttg aaacataaag 1664

agatgtgtac tataataatg taattactat gccctgagaa aatcactgct gaggagctct 1724
 tgteccctctg tgaagatcag taggaaaatg gtggcttggc gtgctgacaa tgatgagcag 1784
 accaactcaa aatttggaataa attaagacca tgaagatgga atcagctctg gatcctggat 1844
 ccacttctat ctgggccctt gctaacctga gaaggatctg cctgcggaac aagctataga 1904
 taagccttag cagagaacac tgggtcaagc actgcatatt gtgaaccacac ttctcttctt 1964
 gaaagaaatg actgagatga tgggtccagag caactatgca agagccaact gagagatcac 2024
 aacactcaaa agagaaaaaa aatgaaagat cttgacaaca gagatgcata tgaatgtcct 2084
 gtctgtccag tcctctgaca aaccttggga ttagcaacag gtagacagtc tgtccaaaag 2144
 gacttaagac agacagcagc tcccatggtg gttggtgaga agtttgata ataatacaagt 2204
 tattgtgatg ttctatctgg ctgcaggcag agcaggggag gaagagctat catcttgata 2264
 atgggataaa tggaaggaa cttaggactc tttcaactta cttctgagac acaaagagct 2324
 agagtggaaat caggaggacc aagatgtaaa tcatctaaag gccaaacttag ctggcagggtg 2384
 ccctagggag agatcagctg gccaaagagca ggggtggcaa atttatatct gaggactgtc 2444
 tatatgtcat tataaatgat ggggaaactg ggtacacgag aggactatac tagcccagta 2504
 gagatgagtc agatgaccct ggctctaaag cagcatcact aaggagcag gcagcctcag 2564
 tccaaaccaa gagagaactt tgggaggccc aaccacaga aatatttctt cttattctac 2624
 ataaaggaat ctagaagggt gtagacagct gtactagcca tgcttgcctt tgtaatatta 2684
 ccatttgtgt gttcttcctt gcatttgctt cattaggcca taagcatctt gttggtttct 2744
 aacatgtttc gtatagttag ttcttaataa attttctta aattgaaaaa aaaaaaaaaa 2804
 aaaaaaaaaa aaaaaaaaaa aaaaaa 2830

<210> 26

<211> 332

<212> PRT

<213> Felis catus

<400> 26

Met Gly Ile Cys Asp Ser Thr Met Gly Leu Ser His Thr Leu Leu Val
 1 5 10 15
 Met Ala Leu Leu Leu Ser Gly Val Ser Ser Met Lys Ser Gln Ala Tyr
 20 25 30
 Phe Asn Lys Thr Gly Glu Leu Pro Cys His Phe Thr Asn Ser Gln Asn
 35 40 45
 Ile Ser Leu Asp Glu Leu Val Val Phe Trp Gln Asp Gln Asp Lys Leu
 50 55 60
 Val Leu Tyr Glu Ile Phe Arg Gly Lys Glu Asn Pro Gln Asn Val His
 65 70 75 80
 Leu Lys Tyr Lys Gly Arg Thr Ser Phe Asp Lys Asp Asn Trp Thr Leu
 85 90 95
 Arg Leu His Asn Val Gln Ile Lys Asp Lys Gly Thr Tyr His Cys Phe
 100 105 110
 Ile His Tyr Lys Gly Pro Lys Gly Leu Val Pro Met His Gln Met Ser
 115 120 125
 Ser Asp Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Thr Val
 130 135 140
 Thr Ser Asn Arg Thr Glu Asn Ser Gly Ile Ile Asn Leu Thr Cys Ser
 145 150 155 160
 Ser Ile Gln Gly Tyr Pro Glu Pro Lys Glu Met Tyr Phe Gln Leu Asn
 165 170 175
 Thr Glu Asn Ser Thr Thr Lys Tyr Asp Thr Val Met Lys Lys Ser Gln
 180 185 190
 Asn Asn Val Thr Glu Leu Tyr Asn Val Ser Ile Ser Leu Pro Phe Ser
 195 200 205
 Val Pro Glu Ala His Asn Val Ser Val Phe Cys Ala Leu Lys Leu Glu
 210 215 220
 Thr Leu Glu Met Leu Leu Ser Leu Pro Phe Asn Ile Asp Ala Gln Pro
 225 230 235 240
 Lys Asp Lys Asp Pro Glu Gln Gly His Phe Leu Trp Ile Ala Ala Val
 245 250 255

Leu Val Met Phe Val Val Phe Cys Gly Met Val Ser Phe Lys Thr Leu
 260 265 270

Arg Lys Arg Lys Lys Lys Gln Pro Gly Pro Ser His Glu Cys Glu Thr
 275 280 285

Ile Lys Arg Glu Arg Lys Glu Ser Lys Gln Thr Asn Glu Arg Val Pro
 290 295 300

Tyr His Val Pro Glu Arg Ser Asp Glu Ala Gln Cys Ile Asn Ile Leu
 305 310 315 320

Lys Thr Ala Ser Gly Asp Lys Ser Thr Thr His Phe
 325 330

<210> 27

<211> 2830

<212> DNA

<213> Felis catus

<400> 27

tttttttttt tttttttttt tttttttttt tttttttttt tcaatttaag aaaaatttat 60
 taagaactca ctatacgaaa catgttagaa accaacaaga tgcttatggc ctaatgaagc 120
 aaatgcaggg aagaacacac aaatggtaat attacaagga caagcatggc tagtacagct 180
 gtctacaacc ttctagattc ctttatgtag aataagaaga aatatctctg tgggttgggc 240
 ctcccaaagt tctctcttgg ttggactga ggctgcctcg tcccttagtg atgctgcttt 300
 agagccaggg tcatctgact catctctact gggctagtat agtctctctg tgtaccagc 360
 ttcccatca ttataatga catatagaca gtctcagat ataaatttgc caccctgct 420
 cttggccagc tgatctctcc ctagggcacc tgccagctaa gttggccttt agatgattta 480
 catcttggtc ctctgattc cactctagct ctttgtgtct cagaagtaag ttgaaagagt 540
 cctaagcttc cttccattta tccattatc aagatgatag ctcttctctc cctgctctgc 600
 ctgcagccag atgaaacatc acaataactt gattattatc caaacttctc accaaccacc 660
 atgggagctg ctgtctgtct taagtccttt tggacagact gtctacctgt tgctaattccc 720
 aaggtttgtc agaggactgg acagacagga cattcatatg catctctgtt gtcaagatct 780

ttcatttttt ttctcttttg agtgtgtgta tctctcagtt ggctcttgca tagttgctct 840
 ggaccatcat ctcagtcatt tctttcaaga agagaagtgg gttcacaata tgcagtgcctt 900
 gacccagtgt tctctgctaa ggcttatcta tagcttgctc cgcaggcaga tcttctcag 960
 gttagcaagg gccagatag aagtggatcc aggatccaga gctgattcca tcttcattggt 1020
 ctttaattttc caaattttga gttggtctgc tcatcattgt cagcaagcca agccaccatt 1080
 ttctactga tcttcacaga gggacaagag ctctcagca gtgattttct cagggcatag 1140
 taattacatt attatagtag acatctcttt atgtttcaag atattaatag caatactata 1200
 aaagccataa aatatataac tcccacaaga ctgaagaggt agttattcag tcacaaatat 1260
 acaagataga atgaggcact accttagaat tacatagata aaagccatta ttccagtaat 1320
 tgggagttct acataagacg caatttataa aattaagaaa agaaggctga atgtgaaaca 1380
 ggaggtgtta gtcttaaaca ttattcctga gccaggatcc agtcagggtga gcttggcgca 1440
 caaatttgct ctgaaatgaa aagaagcagt taaggctctgc tctgaggta gggagcagag 1500
 ctggcattac aggggtggctt tctcagatgg agtcctggaa cctccactat tattaatggt 1560
 aataataata ttaatagaca aaaaaaggta agccaaaac ttgaagggaaggatataa 1620
 acaatggaca gttatatgga ctttattctt taattaaataa tgtgtagtac tttgtcgcc 1680
 tgaggctgtc ttcaaatgt taatacactg ggcttcatca gatctctcag gtacgtggta 1740
 tggtagctctt tgggtggtct gtttgcctc tttctctcc cttttgatgg tttcacattc 1800
 atgagagggg ccaggctgct tcttcttctt tttcttagt gttttaaaagg acaccatccc 1860
 acaaaaaaca acaaacatta caagtagcgc cgcaatccag aggaagtggc cttgttcagg 1920
 gtctttatcc ttaggtgtg catctatatt gaaaggtagg gagagcagca tctccagtgt 1980
 ctccagtttc agggcacaaa agacgtcac attgtgtgct tcagggactg aaaaaggcaa 2040
 gctgatagaa acgttgtaca gttctgtcac attattttga gatttcttca tgacagtatc 2100
 atacttagta gttgaattct cagtgtttag ctgaaaatac atctccttag gttctgggta 2160
 acctgtata gatgagcagg tcaaatttat gatgccagaa tttctgttc tattagaagt 2220

tactgttatt tcaggttgac tgaagttagc aagcactgat aggtcagaac tcatttggtg 2280
catgggaact agtcctttgg gccctttata atgaatgaaa cagtgatatg tggccttgtc 2340
cttgatctga acattgtgga gtctcagggc ccagttgtcc ttgtcaaagc ttgtacggcc 2400
cttatatttg agatgaacat tttgaggggt ctctttgctt ctgaatatct catacagaac 2460
cagcttatcc tggctctgcc aaaatactac cagctcatcc aggcttatgt tttgagagtt 2520
tgtaaaatgg catggcagtt ctccagtctt gttgaaatat gcttgactct tcatggaaga 2580
aacaccagag agcaggaggg ccatcacaag gagagtgtga ctcagtccca tagtgctgtc 2640
acaaatgcc atccactgca gctccagag accagatgta taagctcagt gacattcccg 2700
aggaacacag aaacaagggtg ttaagaataa acgatgagga gcctttgtga gctccccata 2760
acttcctaac actctttgta gagaaagaaa taatgtcagg gctgagacta gaactcaaaa 2820
aaaaaaaaac 2830

<210> 28
<211> 996
<212> DNA
<213> Felis catus

<400> 28
atgggcattt gtgacagcac tatgggactg agtcacactc tccttgtgat ggccctcctg 60
ctctctggtg tttcttccat gaagagtcaa gcatatttca acaagactgg agaactgcc 120
tgccatttta caaactctca aaacataagc ctggatgagc tggtagtatt ttggcaggac 180
caggataagc tggttctgta tgagatattc agaggcaaag agaaccctca aaatgttcat 240
ctcaaataata agggccgtac aagctttgac aaggacaact ggaccctgag actccacaat 300
gttcagatca aggacaaggg cacatatcac tgtttcattc attataaagg gcccaaagga 360
ctagttccca tgcaccaa at gattctgac ctatcagtgc ttgctaactt cagtcaacct 420
gaaataacag taacttctaa tagaacagaa aattctggca tcataaattt gacctgctca 480
tctatacaag gttaccaga acctaggag atgtattttc agctaaacac tgagaattca 540
actactaagt atgatactgt catgaagaaa tctcaaaata atgtgacaga actgtacaac 600

gtttctatca gcttgccctt ttcagtcctt gaagcacaca atgtgagcgt cttttgtgcc 660
 ctgaaactgg agacaactgga gatgctgctc tccctacctt tcaatataga tgcacaacct 720
 aaggataaag accctgaaca aggccacttc ctctggattg cggctgtact tgtaatgttt 780
 gttgtttttt gtgggatggg gtcctttaaa acactaagga aaaggaagaa gaagcagcct 840
 ggcccccttc atgaatgtga aaccatcaaa agggagagaa aagagagcaa acagaccaac 900
 gaaagagtac cataccacgt acctgagaga tctgatgaag cccagtgtat taacattttg 960
 aagacagcct caggcgacaa aagtactaca cttttt 996

<210> 29
 <211> 996
 <212> DNA
 <213> Felis catus

<400> 29
 aaaatgtgta gtacttttgt cgctgaggc tgtcttcaaa atgttaatac actgggcttc 60
 atcagatctc tcaggtaagt ggtatggtac tcttcgttg gtctgtttgc tctcttttct 120
 ctcccttttg atggtttcac attcatgaga ggggccaggc tgcttcttct tcttttctct 180
 tagtgtttta aaggacacca tcccacaaa aacaacaaac attacaagta cagccgcaat 240
 ccagaggaag tggccttggt cagggtcttt atccttaggt tgtgcatcta tattgaaagg 300
 tagggagagc agcatctcca gtgtctccag tttcagggca caaaagacgc tcacattgtg 360
 tgcttcaggg actgaaaaag gcaagctgat agaaacgttg tacagttctg tcacattatt 420
 ttgagatttc ttcatgacag tatcatactt agtagttgaa ttctcagtg ttagctgaaa 480
 atacatctcc ttaggttctg ggtaaccttg tatagatgag cagggtcaat ttatgatgcc 540
 agaattttct gttctattag aagttactgt tatttcaggt tgactgaagt tagcaagcac 600
 tgataggtca gaactcattt ggtgcatggg aactagtcct ttgggccctt tataatgaat 660
 gaaacagtga tatgtgccct tgccttgat ctgaacattg tggagtctca ggtccagtt 720
 gtccttgta aagcttgta ggccttata ttgagatga acattttgag ggttctcttt 780

gcctctgaat atctcataca gaaccagctt atcctgggcc tgccaaaata ctaccagctc 840
 atccaggctt atgttttgag agtttgtaaa atggcatggc agttctccag tcttggtgaa 900
 atatgcttga ctcttcattg aagaaacacc agagagcagg agggccatca caaggagagt 960
 gtgactcagt cccatagtgc tgtcacaaat gcccat 996

<210> 30

<211> 509

<212> DNA

<213> Felis catus

<220>

<221> CDS

<222> (1)..(507)

<400> 30

ata caa ggt tac cca gaa cct aag gag atg tat ttt cag cta aac act 48
 Ile Gln Gly Tyr Pro Glu Pro Lys Glu Met Tyr Phe Gln Leu Asn Thr
 1 5 10 15

gag aat tca act act aag tat gat act gtc atg aag aaa tct caa aat 96
 Glu Asn Ser Thr Thr Lys Tyr Asp Thr Val Met Lys Lys Ser Gln Asn
 20 25 30

aat gtg aca gaa ctg tac aac gtt tct atc agc ttg cct ttt tca gtc 144
 Asn Val Thr Glu Leu Tyr Asn Val Ser Ile Ser Leu Pro Phe Ser Val
 35 40 45

cct gaa gca cac aat gtg agc gtc ttt tgt gcc ctg aaa ctg gag aca 192
 Pro Glu Ala His Asn Val Ser Val Phe Cys Ala Leu Lys Leu Glu Thr
 50 55 60

ctg gag atg ctg ctg tcc cta cct ttc aat ata gat gca caa cct aag 240
 Leu Glu Met Leu Leu Ser Leu Pro Phe Asn Ile Asp Ala Gln Pro Lys
 65 70 75 80

gat aaa gac cct gaa caa ggc cac ttc ctg tgg att gcg gct gta ctt 288
 Asp Lys Asp Pro Glu Gln Gly His Phe Leu Trp Ile Ala Ala Val Leu
 85 90 95

gta atg ttt gtt gtt ttt tgt ggg atg gtg tcc ttt aaa aca cta agg 336
 Val Met Phe Val Val Phe Cys Gly Met Val Ser Phe Lys Thr Leu Arg
 100 105 110

aaa agg aag aag aag cag cct ggc ccc tct cat gaa tgt gaa acc atc 384

Lys Arg Lys Lys Lys Gln Pro Gly Pro Ser His Glu Cys Glu Thr Ile
 115 120 125

aaa agg gag aga aaa gag agc aaa cag acc aac gaa aga gta cca tac 432
 Lys Arg Glu Arg Lys Glu Ser Lys Gln Thr Asn Glu Arg Val Pro Tyr
 130 135 140

cac gta cct gag aga tct gat gaa gcc cag tgt att aac att ttg aag 480
 His Val Pro Glu Arg Ser Asp Glu Ala Gln Cys Ile Asn Ile Leu Lys
 145 150 155 160

aca gcc tca ggc gac aaa agt act aca ca 509
 Thr Ala Ser Gly Asp Lys Ser Thr Thr
 165

<210> 31

<211> 169

<212> PRT

<213> Felis catus

<400> 31

Ile Gln Gly Tyr Pro Glu Pro Lys Glu Met Tyr Phe Gln Leu Asn Thr
 1 5 10 15

Glu Asn Ser Thr Thr Lys Tyr Asp Thr Val Met Lys Lys Ser Gln Asn
 20 25 30

Asn Val Thr Glu Leu Tyr Asn Val Ser Ile Ser Leu Pro Phe Ser Val
 35 40 45

Pro Glu Ala His Asn Val Ser Val Phe Cys Ala Leu Lys Leu Glu Thr
 50 55 60

Leu Glu Met Leu Leu Ser Leu Pro Phe Asn Ile Asp Ala Gln Pro Lys
 65 70 75 80

Asp Lys Asp Pro Glu Gln Gly His Phe Leu Trp Ile Ala Ala Val Leu
 85 90 95

Val Met Phe Val Val Phe Cys Gly Met Val Ser Phe Lys Thr Leu Arg
 100 105 110

Lys Arg Lys Lys Lys Gln Pro Gly Pro Ser His Glu Cys Glu Thr Ile
 115 120 125

Lys Arg Glu Arg Lys Glu Ser Lys Gln Thr Asn Glu Arg Val Pro Tyr
 130 135 140

His Val Pro Glu Arg Ser Asp Glu Ala Gln Cys Ile Asn Ile Leu Lys
 145 150 155 160

Thr Ala Ser Gly Asp Lys Ser Thr Thr
 165

<210> 32
 <211> 509
 <212> DNA
 <213> Felis catus

<400> 32
 tgtgtagtac ttttgtcgcc tgaggctgtc ttcaaaatgt taatacactg ggcttcatca 60
 gatctctcag gtacgtggta tgggtactctt tegtgtgtct gtttgccttc tttctctccc 120
 cttttgatgg ttccacattc atgagagggg ccaggctgct tcttcttccc tttccttagt 180
 gttttaaaagg acaccatccc acaaaaaaca acaaacatta caagtacagc cgcaatccag 240
 aggaagtggc cttgttcagg gtctttatcc ttaggttgtg catctatatt gaaaggtagg 300
 gagagcagca tctccagtgt ctccagtttc agggcacaaa agacgctcac attgtgtgct 360
 tcagggactg aaaaaggcaa gctgatagaa acgttgtaca gttctgtcac attattttga 420
 gatttcttca tgacagtatc atacttagta gttgaattct cagtgtttag ctgaaaatac 480
 atctccttag gttctgggta accttgat 509

<210> 33
 <211> 359
 <212> DNA
 <213> Felis catus

<220>
 <221> CDS
 <222> (1)..(357)

<400> 33
 ata caa ggt tac cca gaa cct aag gag atg tat ttt cag cta aac act 48
 Ile Gln Gly Tyr Pro Glu Pro Lys Glu Met Tyr Phe Gln Leu Asn Thr
 1 5 10 15
 gag aat tca act act aag tat gat act gtc atg aag aaa tct caa aat 96

Glu Asn Ser Thr Thr Lys Tyr Asp Thr Val Met Lys Lys Ser Gln Asn
 20 25 30

 aat gtg aca gaa ctg tac aac gtt tct atc agc ttg cct ttt tca gtc 144
 Asn Val Thr Glu Leu Tyr Asn Val Ser Ile Ser Leu Pro Phe Ser Val
 35 40 45

 cct gaa gca cac aat gtg agc gtc ttt tgt gcc ctg aaa ctg gag aca 192
 Pro Glu Ala His Asn Val Ser Val Phe Cys Ala Leu Lys Leu Glu Thr
 50 55 60

 ctg gag atg ctg ctc tcc cta cct ttc aat ata gaa acc atc aaa agg 240
 Leu Glu Met Leu Leu Ser Leu Pro Phe Asn Ile Glu Thr Ile Lys Arg
 65 70 75 80

 gag aga aaa gag agc aaa cag acc aac gaa aga gta cca tac cac gta 288
 Glu Arg Lys Glu Ser Lys Gln Thr Asn Glu Arg Val Pro Tyr His Val
 85 90 95

 cct gag aga tct gat gaa gcc cag tgt att aac att ttg aag aca gcc 336
 Pro Glu Arg Ser Asp Glu Ala Gln Cys Ile Asn Ile Leu Lys Thr Ala
 100 105 110

 tca ggc gac aaa agt act aca ca 359
 Ser Gly Asp Lys Ser Thr Thr
 115

<210> 34
 <211> 119
 <212> PRT
 <213> Felis catus

<400> 34
 Ile Gln Gly Tyr Pro Glu Pro Lys Glu Met Tyr Phe Gln Leu Asn Thr
 1 5 10 15

 Glu Asn Ser Thr Thr Lys Tyr Asp Thr Val Met Lys Lys Ser Gln Asn
 20 25 30

 Asn Val Thr Glu Leu Tyr Asn Val Ser Ile Ser Leu Pro Phe Ser Val
 35 40 45

 Pro Glu Ala His Asn Val Ser Val Phe Cys Ala Leu Lys Leu Glu Thr
 50 55 60

 Leu Glu Met Leu Leu Ser Leu Pro Phe Asn Ile Glu Thr Ile Lys Arg
 65 70 75 80

Glu Arg Lys Glu Ser Lys Gln Thr Asn Glu Arg Val Pro Tyr His Val
 85 90 95

Pro Glu Arg Ser Asp Glu Ala Gln Cys Ile Asn Ile Leu Lys Thr Ala
 100 105 110

Ser Gly Asp Lys Ser Thr Thr
 115

<210> 35

<211> 359

<212> DNA

<213> Felis catus

<400> 35

tgtgtagtac ttttgtcgcc tgaggtgtgc ttcaaaatgt taatacactg ggcttcatca 60
 gatctctcag gtacgtggta tgggtactctt tcgttgggtct gtttgcctctc ttttctctcc 120
 cttttgatgg tttctatatt gaaaggtagg gagagcagca tctccagtgt ctccagtttc 180
 agggcacaaa agacgctcac atttgtgtgt tcagggactg aaaaaggcaa gctgatagaa 240
 acgttgtaca gttctgtcac attattttga gatttcttca tgacagtatc atacttagta 300
 gttgaattct cagtgttttag ctgaaaatac atctccttag gttctgggta accttgat 359

<210> 36

<211> 594

<212> DNA

<213> Felis catus

<220>

<221> CDS

<222> (1)..(522)

<400> 36

atg ggt cac gca gca aag tgg aaa aca cca cta ctg aag cac cca tat 48
 Met Gly His Ala Ala Lys Trp Lys Thr Pro Leu Leu Lys His Pro Tyr
 1 5 10 15
 ccc aag ctc ttt ccg ctc ttg atg cta gct agt ctt ttt tac ttc tgt 96
 Pro Lys Leu Phe Pro Leu Leu Met Leu Ala Ser Leu Phe Tyr Phe Cys
 20 25 30

tca ggt atc atc cag gtg aac aag aca gtg gaa gaa gta gca gta cta 144
Ser Gly Ile Ile Gln Val Asn Lys Thr Val Glu Glu Val Ala Val Leu
35 40 45

tcc tgt gat tac aac att tcc acc aaa gaa ctg acg gaa att cga atc 192
Ser Cys Asp Tyr Asn Ile Ser Thr Lys Glu Leu Thr Glu Ile Arg Ile
50 55 60

tat tgg caa aag gat gat gaa atg gtg ttg gct gtc atg tct ggc aaa 240
Tyr Trp Gln Lys Asp Asp Glu Met Val Leu Ala Val Met Ser Gly Lys
65 70 75 80

gta caa gtg tgg ccc aag tac aag aac cgc aca ttc act gac gtc acc 288
Val Gln Val Trp Pro Lys Tyr Lys Asn Arg Thr Phe Thr Asp Val Thr
85 90 95

gat aac cac tcc att gtg atc atg gct ctg cgc ctg tca gac aat ggc 336
Asp Asn His Ser Ile Val Ile Met Ala Leu Arg Leu Ser Asp Asn Gly
100 105 110

aaa tac act tgt att att caa aag att gaa aaa ggg tct tac aaa gtg 384
Lys Tyr Thr Cys Ile Ile Gln Lys Ile Glu Lys Gly Ser Tyr Lys Val
115 120 125

aaa cac ctg act tcg gtg atg tta ttg gtc aga ggc gtc aca ccc agc 432
Lys His Leu Thr Ser Val Met Leu Leu Val Arg Gly Val Thr Pro Ser
130 135 140

aca gag ccc aat gcc cat gcg gag ctt gaa atc atg acc ctg aga tca 480
Thr Glu Pro Asn Ala His Ala Glu Leu Glu Ile Met Thr Leu Arg Ser
145 150 155 160

aga cct gag ctg aga tca aga gtc gga cgc tta atc gac tga 522
Arg Pro Glu Leu Arg Ser Arg Val Gly Arg Leu Ile Asp
165 170

gccacccagg catcccaatg atactttcta aataaactct taaaaaaaaa aaaaaaaaaa 582

aaaaaaaaaa aa 594

<210> 37

<211> 173

<212> PRT

<213> Felis catus

<400> 37

Met Gly His Ala Ala Lys Trp Lys Thr Pro Leu Leu Lys His Pro Tyr


```
<210> 38
<211> 594
<212> DNA
<213> Felis catus
```

44

gttatcggtg acgtcagtga atgtgcggtt cttgtacttg ggccacactt gtactttgcc 360
 agacatgaca gccaacacca tttcatcatc cttttgccaa tagattcgaa tttccgtcag 420
 ttcttttggtg gaaatggtgt aatcacagga tagtactgct acttcttcca ctgtcttggt 480
 cacctggatg atacctgaac agaagtaaaa aagactagct agcatcaaga gcggaaagag 540
 cttgggatat ggggtgcttca gtagtgggtg tttccacttt gctgcgtgac ccat 594

<210> 39

<211> 519

<212> DNA

<213> Felis catus

<400> 39

atgggtcacg cagcaaagtg gaaaacacca ctactgaagc accatatcc caagctcttt 60
 ccgctcttga tgctagctag tcttttttac ttctgttcag gtatcatcca ggtgaacaag 120
 acagtggaag aagtagcagt actatcctgt gattacaaca tttccaccaa agaactgacg 180
 gaaattcgaa tctattggca aaaggatgat gaaatggtgt tggctgtcat gtctggcaaa 240
 gtacaagtgt ggcccaagta caagaaccgc acattcactg acgtcaccga taaccactcc 300
 attgtgatca tggctctgcg cctgtcagac aatggcaa at acacttgtat tattcaaaag 360
 attgaaaaag ggtcttataa agtgaaacac ctgacttcgg tgatgttatt ggtcagaggg 420
 gtcacacca gcacagagcc caatgcccat gggagcttg aaatcatgac cctgagatca 480
 agacctgagc tgagatcaag agtcggacgc ttaatcgac 519

<210> 40

<211> 519

<212> DNA

<213> Felis catus

<400> 40

gtcgattaag cgtecgactc ttgatctcag ctcaggtctt gatctcaggg tcatgatttc 60
 aagctccgca tgggcattgg gctctgtgct ggggtgtgacg cctctgacca ataacatcac 120
 cgaagtcagg tgtttcactt tgtaagaccc tttttcaatc ttttgaataa tacaagtgt 180

tttgccattg tctgacaggc gcagagccat gatcacaatg gagtgggttat cggtgacgtc 240
 agtgaatgtg cggttcttgt acttggggcca cacttgtaact ttgccagaca tgacagccaa 300
 caccatttca tcatcctttt gccaatagat tcgaatttcc gtcagttctt tgggtggaaat 360
 gttgtaatca caggatagta ctgctacttc ttccactgtc ttgttcacct ggatgatacc 420
 tgaacagaag taaaaaagac tagctagcat caagagcggg aagagcttgg gatatgggtg 480
 cttcagtagt ggtgttttcc actttgctgc gtgacccat 519

<210> 41

<211> 1856

<212> DNA

<213> Canis familiaris

<220>

<221> CDS

<222> (60)..(731)

<400> 41

caggatcctg aaaggtttca ctctgcttcc tgaagacctg aacactgtct cataaagcc 59
 atg gct ggc ttt gga ttc cgg agg cat ggg gct cag ccg gac ctg gct 107
 Met Ala Gly Phe Gly Phe Arg Arg His Gly Ala Gln Pro Asp Leu Ala
 1 5 10 15
 tct agg acc tgg ccc tgc act gct ctg ttt tct ctt ctc ttt atc ccc 155
 Ser Arg Thr Trp Pro Cys Thr Ala Leu Phe Ser Leu Leu Phe Ile Pro
 20 25 30
 gtc ttc tcc aaa ggg atg cat gtg gct cag cct gca gtg gtt ctg gcc 203
 Val Phe Ser Lys Gly Met His Val Ala Gln Pro Ala Val Val Leu Ala
 35 40 45
 agc agc cgg ggt gtt gct agc ttc gtg tgt gaa tat ggg tct tca ggc 251
 Ser Ser Arg Gly Val Ala Ser Phe Val Cys Glu Tyr Gly Ser Ser Gly
 50 55 60
 aac gca gcc gag gtc cgg gtg aca gtg ctg cgg cag gct ggc agc cag 299
 Asn Ala Ala Glu Val Arg Val Thr Val Leu Arg Gln Ala Gly Ser Gln
 65 70 75 80
 atg act gaa gtc tgt gcc gcg aca tac aca gtg gag gat gag ttg gcc 347
 Met Thr Glu Val Cys Ala Ala Thr Tyr Thr Val Glu Asp Glu Leu Ala

	85	90	95	
ttc ctg gat gat tct acc tgc acc ggc acc tcc agt gga aac aaa gtg				395
Phe Leu Asp Asp Ser Thr Cys Thr Gly Thr Ser Ser Gly Asn Lys Val				
	100	105	110	
aac ctc acc atc caa ggg ttg agg gcc atg gac acg ggg ctc tac atc				443
Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile				
	115	120	125	
tgc aag gtg gag ctc atg tac cca cca ccc tac tat gta ggc atg gga				491
Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Val Gly Met Gly				
	130	135	140	
aat gga acc cag att tat gtc atc gat cct gaa cct tgc cca gat tct				539
Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser				
	145	150	155	160
gac ttc ctc ctc tgg atc ctt gca gca gtc agt tgc ggc ttg ttt ttt				587
Asp Phe Leu Leu Trp Ile Leu Ala Ala Val Ser Ser Gly Leu Phe Phe				
	165	170	175	
tat agc ttt ctt atc aca gct gtt tct ttg agc aaa atg cta aag aaa				635
Tyr Ser Phe Leu Ile Thr Ala Val Ser Leu Ser Lys Met Leu Lys Lys				
	180	185	190	
aga agc cct ctt acc aca ggg gtc tat gtg aaa atg ccc cca act gag				683
Arg Ser Pro Leu Thr Thr Gly Val Tyr Val Lys Met Pro Pro Thr Glu				
	195	200	205	
cca gaa tgt gaa aag caa ttt cag cct tat ttt att ccc atc aat tga				731
Pro Glu Cys Glu Lys Gln Phe Gln Pro Tyr Phe Ile Pro Ile Asn				
	210	215	220	
gagatcatta tgaagaagaa agaatatattt ccaatttcca ggagctgagg caattctaac 791				
tttgtgtat ccagctatgt gtacttggtt gtatatatttg gggggggtt catctctctt 851				
taatataaag ctggatgcag aacccaaatg aagtgtacta caaattcaaa gcaaagggtc 911				
aagaaaacag agccaggatg tttctgtcac atcagatcca attttcgtaa aagtatcact 971				
tgggagcaat atggggatgc agcattagga catgcgctct aggatatagg ttagggagtg 1031				
gtgcgggtcca aagaaagcaa aggagagaga gtcagggaga ggatgatatt gtacacactt 1091				
tgtatttaca tgtgagaagt ttatagctga agtgacgttt tcaagttaaa tttttgtgct 1151				

atgttatttt tcataaatgt aaaatcacgt gaagacttta aaaatattca catggctata 1211
 ttttagccag tgattccaaa ggttgatttg taccaatata tttttttta tctgatagta 1271
 ttatgcatgg gggccacatg tgcttttggtg tatttggtga tggtttcaat ataaacacta 1331
 tatggcagtg ttttcccacc aggggctcag gggaagtttt atggagggat tcaggacact 1391
 aatacgccag gtaaaataca aggtcacttg gtaactggct tggaaactgg atgaggtcat 1451
 agttgattct tgtagacgtg ttgggctaaa ttggtgttga catgtgcttt gggcttttat 1511
 gttagctcct ttcaaagatt tgtaaggag tcaaaactgg tatatctgat ttaactccat 1571
 agaacaccat cgtcaagtaa acggctcatt ccaggagtct tggaggtatg aacttcaagg 1631
 aagctctagt ttcacaaggg cccaattcc ttgctcatgg ttaatgccat gggcagaaaa 1691
 cagcagcagg tggcagaaca ggtgatgaa ggtttccgaa aacaaacact gttggtgttt 1751
 ttttaactca ctattttctg tgaaaatgca acaacatgta taatattttt aattaaataa 1811
 aaatctgtgg tggtcattaa aaaaaaaaaa aaaaaaaaaa aaaaaa 1856

<210> 42

<211> 223

<212> PRT

<213> Canis familiaris

<400> 42

Met Ala Gly Phe Gly Phe Arg Arg His Gly Ala Gln Pro Asp Leu Ala
 1 5 10 15

Ser Arg Thr Trp Pro Cys Thr Ala Leu Phe Ser Leu Leu Phe Ile Pro
 20 25 30

Val Phe Ser Lys Gly Met His Val Ala Gln Pro Ala Val Val Leu Ala
 35 40 45

Ser Ser Arg Gly Val Ala Ser Phe Val Cys Glu Tyr Gly Ser Ser Gly
 50 55 60

Asn Ala Ala Glu Val Arg Val Thr Val Leu Arg Gln Ala Gly Ser Gln
 65 70 75 80

Met Thr Glu Val Cys Ala Ala Thr Tyr Thr Val Glu Asp Glu Leu Ala
 85 90 95

Phe Leu Asp Asp Ser Thr Cys Thr Gly Thr Ser Ser Gly Asn Lys Val
100 105 110

Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
115 120 125

Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Val Gly Met Gly
130 135 140

Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser
145 150 155 160

Asp Phe Leu Leu Trp Ile Leu Ala Ala Val Ser Ser Gly Leu Phe Phe
165 170 175

Tyr Ser Phe Leu Ile Thr Ala Val Ser Leu Ser Lys Met Leu Lys Lys
180 185 190

Arg Ser Pro Leu Thr Thr Gly Val Tyr Val Lys Met Pro Pro Thr Glu
195 200 205

Pro Glu Cys Glu Lys Gln Phe Gln Pro Tyr Phe Ile Pro Ile Asn
210 215 220

<210> 43

<211> 1856

<212> DNA

<213> Canis familiaris

<400> 43

tttttttttt tttttttttt tttttttaat gaccaccaca gatttttatt taattaaaaa 60
tattatacat gttgttgcac tttcacagaa aatagtgcag taaaaaaaca ccaacagtgt 120
ttgttttcgg aaaccttcac caccctgttc tgccacctgc tgcgtttttc tgcccatggc 180
attaaccatg agcaaggaat tggggccctt gtgaaactag agcttccttg aagttcatatc 240
ctccaagact cctggaatga gccgtttact tgacgatggt gttctatgga gttaaatacag 300
atataccagt ttgactccc ttacaaatct ttgaaaggag ctaacataaa agcccaaagc 360
acatgtcaac accaatttag cccaacacgt ctacaagaat caactatgac ctcatccagt 420
ttccaagcca gttaccaagt gaccttgtat tttaacctggc gtattagtgt cctgaatccc 480

tccataaaac ttcccctgag cccctggtgg gaagacactg ccatatagtg tttatattga 540
 aaccatcaac aaatacacaa aagcacatgt ggcccccatg cataatacta tcagataaaa 600
 aaatatatat tgggtacaata caacctttgg aatcactggc taaaatatag ccatgtgaat 660
 atttttaaag tcttcacgtg attttacatt tatgaaaaat aacatagcac aaaaatttaa 720
 cttgaaaacg tcacttcagc tataaacttc tcacatgtaa atacaaagtg tgtacaatat 780
 catcctctcc ctgactctct ctcccttgct ttctttggac cgcaccactc cctaacctat 840
 atcctagagc gcatgtccta atgctgcac cccatattgc tcccaagtga tacttttaag 900
 aaaattggat ctgatgtgac agaaacatcc tggtctgttt ttcttgacc tttgctttga 960
 attttagta cacttcattt gggttctgca tccagcttta tattaaagag agatgaaacc 1020
 cccccaaaa tatacaaaca agtacacata gctggatagc acaaagttag aattgcctca 1080
 gtcctggaa attgaaaaat attctttctt cttcataatg atctctcaat tgatgggaat 1140
 aaaataaggc tgaaattgct tttcacattc tggctcagtt gggggcattt tcacatagac 1200
 cctgtggta agagggcttc tttcttttag cattttgctc aaagaaacag ctgtgataag 1260
 aaagctataa aaaaacaagc ccgaactgac tgctgcaagg atccagagga ggaagtcaga 1320
 atctgggcaa gggtcaggat cgatgacata aatctgggtt ccatttccca tgctacata 1380
 gtagggtggt gggtacatga gctccacctt gcagatgtag agccccgtgt ccatggccct 1440
 caacccttgg atggtgaggt tcactttggt tccactggag gtgccggtgc aggtagaatc 1500
 atccaggaag gccaaactcat cctccactgt gtatgtcgcg gcacagactt cagtcacttg 1560
 gctgccagcc tgcgcagca ctgtcaccgg gacctcggt gcgttgctg aagaccata 1620
 ttcacacacg aagctagcaa caccocggt gctggccaga accactgcag gctgagccac 1680
 atgcatccct ttggagaaga cggggataaa gagaagagaa aacagagcag tgcagggcca 1740
 ggtcctagaa gccaggtccg gctgagcccc atgctccgg aatccaaagc cagccatggc 1800
 tttatggagc agtggtcagg tcttcaggaa gcagagtga acctttcagg atcctg 1856

<210> 44

<211> 669
 <212> DNA
 <213> Canis familiaris

<400> 44

atggctggct ttggattccg gaggcacggg gctcagccgg acctggcttc taggacctgg 60
 ccctgcaactg ctctgttttc tcttctcttt atccccgtct tctccaaagg gatgcatgtg 120
 gctcagcccg cagtgggtct ggccagcagc cgggggtgtg ctagcttcgt gtgtgaatat 180
 ggggtcttcag gcaacgcagc cgaggtcggg gtgacagtgc tgcggcaggc tggcagccag 240
 atgactgaag tctgtgccgc gacatacaca gtggaggatg agttggcctt cctggatgat 300
 tctacctgca ccggcacctc cagtggaaac aaagtgaacc tcaccatcca agggttgagg 360
 gccatggaca cggggctcta catctgcaag gtggagctca tgtaccacc accctactat 420
 gtaggcacgg gaaatggaac ccagatttat gtcacgatc ctgaaccttg ccagattct 480
 gacttctcc tctggatcct tgcagcagtc agttcgggct tgttttttta tagctttctt 540
 atcacagctg tttctttgag caaatgcta aagaaaagaa gccctcttac cacaggggtc 600
 tatgtgaaaa tgccccaac tgagccagaa tgtgaaaagc aatttcagcc ttattttatt 660
 cccatcaat 669

<210> 45
 <211> 669
 <212> DNA
 <213> Canis familiaris

<400> 45

attgatggga ataaaataag gctgaaattg cttttcacat tctggctcag ttgggggcat 60
 tttcacatag acccctgtgg taagagggtt tcttttcttt agcattttgc tcaaagaaac 120
 agctgtgata agaaagctat aaaaaaaca gcccgaaactg actgctgcaa ggatccagag 180
 gaggaagtca gaatctgggc aagggttcagg atcgatgaca taaatctggg ttccatttcc 240
 catgcctaca tagtaggggt gtgggtacat gagctccacc ttgcagatgt agagccccgt 300
 gtccatggcc ctcaaccctt ggatgggtgag gttcactttg ttccactgg aggtgccggg 360

gcaggtagaa tcatccagga aggccaaactc atcctccact gtgtatgtcg cggcacagac 420
 ttcagtcate tggctgccag cctgccgcag cactgtcacc cggacctcgg ctgcgttgcc 480
 tgaagaccca tattcacaca cgaagctagc aacaccccgg ctgctggcca gaaccactgc 540
 aggetgagcc acatgcatcc ctttggagaa gacggggata aagagaagag aaaacagagc 600
 agtgcagggc caggtcctag aagccaggtc cggctgagcc ccatgcctcc ggaatccaaa 660
 gccagccat 669

<210> 46
 <211> 1883
 <212> DNA
 <213> Felis catus

<220>
 <221> CDS
 <222> (69)..(740)

<400> 46
 caaagcttca ggatcctgaa aggtttcact ctgcttctcg aagacctgaa cactgctccc 60
 ataaagcc atg gct tgc ttt gga ttc cgg agg cat ggg gct cag ctg gac 110
 Met Ala Cys Phe Gly Phe Arg Arg His Gly Ala Gln Leu Asp
 1 5 10
 ctg gct tct agg acc tgg ccc tgc act gct ctg ttt tct ctt ctc ttt 158
 Leu Ala Ser Arg Thr Trp Pro Cys Thr Ala Leu Phe Ser Leu Leu Phe
 15 20 25 30
 atc ccc gtc ttc tcc aaa ggg atg cat gtg gcc cag cct gca gtg gtg 206
 Ile Pro Val Phe Ser Lys Gly Met His Val Ala Gln Pro Ala Val Val
 35 40 45
 ctg gcc agc agc cga ggt gtc gcc agc ttc gtg tgt gaa tat ggg tct 254
 Leu Ala Ser Ser Arg Gly Val Ala Ser Phe Val Cys Glu Tyr Gly Ser
 50 55 60
 tca ggc aat gcc gcc gaa gtc cga gtg act gtg ctg agg cag act ggc 302
 Ser Gly Asn Ala Ala Glu Val Arg Val Thr Val Leu Arg Gln Thr Gly
 65 70 75
 agc cag atg act gaa gtc tgt gct gcg aca tac aca gtg gag aat gag 350
 Ser Gln Met Thr Glu Val Cys Ala Ala Thr Tyr Thr Val Glu Asn Glu
 80 85 90

ttg gcc ttc cta gat gat tcc acc tgc act ggc atc tcc agc gga aac 398
 Leu Ala Phe Leu Asp Asp Ser Thr Cys Thr Gly Ile Ser Ser Gly Asn
 95 100 105 110

aaa gtg aac ctc acc atc caa ggg ttg agg gcc atg gac acg gga ctc 446
 Lys Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu
 115 120 125

tac atc tgc aag gtg gag ctc atg tac cca cca ccc tac tat gca ggc 494
 Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Ala Gly
 130 135 140

atg ggc aat gga acc cag att tat gtc atc gat cct gaa cct tgc cca 542
 Met Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro
 145 150 155

gat tct gac ttc ctc ctc tgg atc ctc gca gca gtc agt tca gga ttg 590
 Asp Ser Asp Phe Leu Leu Trp Ile Leu Ala Ala Val Ser Ser Gly Leu
 160 165 170

ttt ttt tat agc ttc ctt atc aca gct gtt tct ttg agc aaa atg cta 638
 Phe Phe Tyr Ser Phe Leu Ile Thr Ala Val Ser Leu Ser Lys Met Leu
 175 180 185 190

aag aaa aga agc cct ctt act aca ggg gtc tat gtg aaa atg ccc cca 686
 Lys Lys Arg Ser Pro Leu Thr Thr Gly Val Tyr Val Lys Met Pro Pro
 195 200 205

aca gag cca gaa tgt gaa aag caa ttt cag cct tat ttt att ccc atc 734
 Thr Glu Pro Glu Cys Glu Lys Gln Phe Gln Pro Tyr Phe Ile Pro Ile
 210 215 220

aat tga cacaccgtta tgaagaagga agaacactgt ccaatttcta agagctgagg 790
 Asn

caattctaac tttttgctat ccagctatgt tgcttatttg tgtatttttg ggggggattc 850

atctctcttt aatataaagc tggatgcaaa atccagatga agtgactac aatttgaagc 910

aaagggtgcag gaaaacagag ccaggatggt tctgtcacat cagatccaat tttagtaaaa 970

gcatcactcg ggagcaatat agggatgcag tcttacgttg taggtgaagg atatgggtta 1030

ggggggtggtg ctgtccaaag aatacaaagg aagagagtta gggagaggat gatattgtac 1090

acactttgta tttacacatg agaagtttat agctgaagtg atgttttcaa gttaaagttt 1150

tgtgctgtta tttttcttaa atgtggaatt acatgaagac tttaaaaata ctcacgtggc 1210
 tatatttttag ccagtgattc caaagggtgt attgtaccaa tatgtatttt tttttatttg 1270
 atagtattgt gcatggggac cacatgtgct tttgtgtatt tgctgatggg tttaatataa 1330
 acactatatg gcagtgtctt cccaccatgg gttcagggga agttttatgg aggggctcag 1390
 gacactaata caccaggtag aacacaaagt cacttggtta ctggcttgga aactggctga 1450
 ggtcataact gattcttata gacacgttga gctgaattgg tgttgacatg tgatttgggc 1510
 ttttatgtta gtcctttca aagggttgca agggagtcca gactgggtga tctgatgtaa 1570
 ttcaatagaa caccaacctc aagaaaatgg ctactccag gggctctgta ggtacgaact 1630
 tcaaggaagc tctagtttca caagggcccc aattcctaac acatggttca tgccatggac 1690
 agaaaaaagc agccggtggc agaacggggg gatgaaagtt tctaaaaact aacactgttg 1750
 gtgtttttta actcattatt ttccatgaaa atgcaacaac atgtataata tttttaatta 1810
 aataaaaaatc tgtggtgggc aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1870
 aaaaaaaaaa aaa 1883

<210> 47

<211> 223

<212> PRT

<213> Felis catus

<400> 47

Met Ala Cys Phe Gly Phe Arg Arg His Gly Ala Gln Leu Asp Leu Ala
 1 5 10 15

Ser Arg Thr Trp Pro Cys Thr Ala Leu Phe Ser Leu Leu Phe Ile Pro
 20 25 30

Val Phe Ser Lys Gly Met His Val Ala Gln Pro Ala Val Val Leu Ala
 35 40 45

Ser Ser Arg Gly Val Ala Ser Phe Val Cys Glu Tyr Gly Ser Ser Gly
 50 55 60

Asn Ala Ala Glu Val Arg Val Thr Val Leu Arg Gln Thr Gly Ser Gln
 65 70 75 80

Met Thr Glu Val Cys Ala Ala Thr Tyr Thr Val Glu Asn Glu Leu Ala
85 90 95

Phe Leu Asp Asp Ser Thr Cys Thr Gly Ile Ser Ser Gly Asn Lys Val
100 105 110

Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
115 120 125

Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Ala Gly Met Gly
130 135 140

Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser
145 150 155 160

Asp Phe Leu Leu Trp Ile Leu Ala Ala Val Ser Ser Gly Leu Phe Phe
165 170 175

Tyr Ser Phe Leu Ile Thr Ala Val Ser Leu Ser Lys Met Leu Lys Lys
180 185 190

Arg Ser Pro Leu Thr Thr Gly Val Tyr Val Lys Met Pro Pro Thr Glu
195 200 205

Pro Glu Cys Glu Lys Gln Phe Gln Pro Tyr Phe Ile Pro Ile Asn
210 215 220

<210> 48

<211> 1883

<212> DNA

<213> Felis catus

<400> 48

tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttgaccacc 60
acagattttt atttaattaa aaatattata catgttggtg cattttcatg gaaaataatg 120
agttaaaaaa caccaacagt gtagtttttt agaaactttc atcaccccggt tctgccaccg 180
gctgcttttt tctgtccatg gcatgaacca tgtgttagga attggggccc ttgtgaaact 240
agagcttcct tgaagttcgt acctacaaga ccctggagt gagccatttt cttgaggttg 300
gtgttctatt gaattacatc agatacacca gtctggactc ccttgcaaac ctttgaaagg 360
agctaacata aaagcccaaa tcacatgtca acaccaattc agctcaacgt gtctataaga 420

atcagttatg acctcagcca gtttccaagc cagttaccaa gtgactttgt gttctacctg 480
 gtgtattagt gtcccgagcc cctccataaa acttcccctg aacccatggt gggaagacac 540
 tgccatatag tgtttatatt aaaaccatca gcaaatcac aaaagcacat gtggtcccca 600
 tgcacaatac tatcaaataa aaaaaaatac atattggtac aatacaacct ttggaatcac 660
 tggctaaaat atagccacgt gagtattttt aaagtcttca tgtaattcca catttaagaa 720
 aaataacagc acaaaacttt aacttgaaaa catcacttca gctataaact tctcatgtgt 780
 aaatacaaag tgtgtacaat atcatcctct ccctaactct ctccctttgt attctttgga 840
 cagcaccacc ccctaacca tacccttcac ctacaacgta agactgcac cctatattgc 900
 tcccgagtga tgcttttact aaaattggat ctgatgtgac agaaacatcc tggctctgtt 960
 ttccctgcacc ttgcttcaa attgtagtac acttcatctg gattttgcat ccagctttat 1020
 attaaagaga gatgaatccc ccccaaaaat acacaaataa gcaacatagc tggatagcaa 1080
 aaagttagaa ttgcctcagc tcttagaaat tggacagtgt tcttccttct tcataacggt 1140
 gtgtcaattg atgggaataa aataaggctg aaattgcttt tcacattctg gctctgttgg 1200
 gggcattttc acatagacc ctagtagtaag agggcttctt ttctttagca ttttgctcaa 1260
 agaaacagct gtgataagga agctataaaa aaacaatcct gaactgactg ctgagaggat 1320
 ccagaggagg aagtcagaat ctgggcaagg ttcaggatcg atgacataaa tctgggttcc 1380
 attgcccatg cctgcatagt aggggtggtg gtacatgagc tccacctg agatgtagag 1440
 tcccggtgac atggccctca acccttggtg ggtgaggttc actttgttcc cgtgagagat 1500
 gccagtgcag gtggaatcat ctaggaaggc caactcattc tccactgtgt atgtcgcagc 1560
 acagacttca gtcactctggc tgccagtctg cctcagcaca gtcactcgga ctccggcggc 1620
 attgcctgaa gacccatatt cacacacgaa gctggcgaca cctcggctgc tggccagcac 1680
 cactgcaggc tgggccacat gcatcccttt ggagaagacg gggataaaga gaagagaaaa 1740
 cagagcagtg cagggccagg tcttagaagc caggctccagc tgagcccat gccctcggaa 1800
 tccaaagcaa gccatggctt tatgggagca gtgttcaggt cttcaggaag cagagtgaag 1860

cctttcagga tcttgaagct ttg

1883

<210> 49

<211> 669

<212> DNA

<213> Felis catus

<400> 49

atggccttgc ttggattccg gaggcattgg gctcagctgg acctggcttc taggacctgg 60

ccctgcaactg ctctgttttc tcttctcttt atccccgtct tctccaaagg gatgcatgtg 120

gccagacctg cagtgggtgc gccagcagc cgaggtgtcg ccagcttcgt gtgtgaatat 180

gggtcttcag gcaatgccgc cgaagtccga gtgactgtgc tgaggcagac tggcagccag 240

atgactgaag tctgtgctgc gacatacaca gtggagaatg agttggcctt cctagatgat 300

tccacctgca ctggcatctc cagcggaaac aaagtgaacc tcaccatcca agggttgagg 360

gccatggaca cgggactcta catctgcaag gtggagctca tgtaoccacc accctactat 420

gcaggcatgg gcaatggaac ccagatttat gtcactgac ctgaaccttg ccagattctt 480

gacttctctc tctggatcct cgcagcagtc agttcaggat tgttttttta tagcttcctt 540

atcacagctg tttctttgag caaaatgcta aagaaaagaa gccctcttac tacaggggtc 600

tatgtgaaaa tgcccccaac agagccagaa tgtgaaaagc aatttcagcc ttattttatt 660

cccatcaat 669

<210> 50

<211> 669

<212> DNA

<213> Felis catus

<400> 50

attgatggga ataaaataag gctgaaattg cttttcacat tctggctctg ttgggggcat 60

tttcacatag acccctgtag taagagggtc tcttttcttt agcattttgc tcaaagaaac 120

agctgtgata aggaagctat aaaaaaacia tcttgaactg actgctgcga ggatccagag 180

gaggaagtca gaatctgggc aaggttcagg atcgatgaca taaatctggg ttccattgcc 240

catgcctgca tagtaggggtg gtgggtacat gagctccacc ttgcagatgt agaqtcccgt 300
gtccatggcc ctcaaccctt ggatgggtgag gttcactttg tttccgctgg agatgccagt 360
gcaggtggaa tcatctagga aggccaactc attctccact gtgtatgtcg cagcacagac 420
ttcagtcate tggctgccag tetgcctcag cacagtcaact cggacttcgg cggcattgcc 480
tgaagaccca tattcacaca cgaagctggc gacacctcgg ctgctggcca gcaccactgc 540
aggctggggc acatgcatcc ctttggagaa gacggggata aagagaagag aaaacagagc 600
agtgcagggc caggtcctag aagccaggtc cagctgagcc ccatgcctcc ggaatccaaa 660
gcaagccat 669

<210> 51

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 51

atacaagggtt acccagaacc 20

<210> 52

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 52

tgtgtagtac ttttgcgcc 20

<210> 53

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 53

gggaattcgc caccatgggt cacgcagcaa agtg

34

<210> 54

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 54

ccctcgagct atgtagacag gtgagatc

28

<210> 55

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 55

gtaatacgac tcactatagg gc

22

<210> 56

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 56

accactccat tgtgatcatg

20

<210> 57

<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 57

gtcttgatct cagggtcatg

20

<210> 58

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 58

gcggatccac catgggcatt tgtgacagca c

31

<210> 59

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 59

gcctcgagtt aaaaatgtgt agtacttttg tcg

33

<210> 60

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 60

gtgaacctsa cyatccaagg

20

<210> 61

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 61

gcattttcac atagaccct g

21

<210> 62

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 62

ggtacgtagg gatgcatgtg gctcagc

27

<210> 63

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 63

ccgaattctc agtcagaatc tgggcaaggt tc

32

<210> 64

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 64

ggtacgtagg tgctgcttcc atgaagag

28

<210> 65

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 65

cccctagggtt aaaactgtgt agtactgttg tcgcc

35